The Molecular Origins of Life:

Replication or Metabolism-First? Advanced Version

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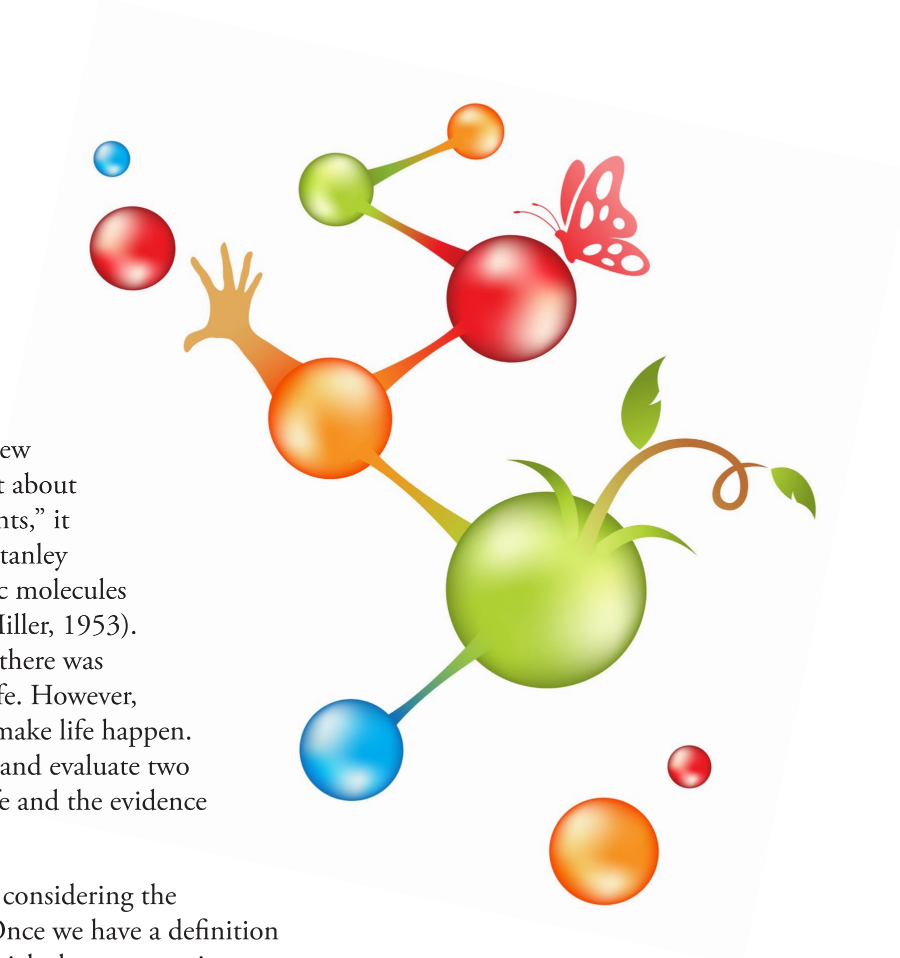
Part I – What Is Life?



Our modern understanding of the origins of life dispels the Aristotelian notion of spontaneous generation, the idea that life arose from inanimate matter. We know that old rags and wheat will not generate adult rats. Louis Pasteur famously showed that organisms will only crop up if parental organisms are initially present in a closed system.

This conclusion applies to the generation of new organisms from parental ones. However, what about the very first life forms? Since it had no "parents," it had to have arisen out of non-living matter. Stanley Miller first demonstrated in 1953 that organic molecules can be created from simple inorganic ones (Miller, 1953). This challenged the common perception that there was something "special" about the molecules of life. However, organic molecules alone are not sufficient to make life happen. How did life originate? Here, we will explore and evaluate two competing hypotheses about the origins of life and the evidence supporting them.

Before we tackle these hypotheses, it is worth considering the deceptively simple question: "What is life"? Once we have a definition for life, we can explore the mechanisms by which these properties first arose. Please brainstorm ideas about the essential characteristics and properties that all living things must have. Do not limit your brainstorming to terrestrial life: what properties would life, anywhere in the universe, have to exhibit for us to consider it alive?

Part II – Gaining Knowledge of One Hypothesis: Jigsaw Instructions

(1) The class will separate into working groups of four members. Within the working groups, assign two members to Team 1 and two members to Team 2. For this first section, Team 1 and Team 2 separate. Team 1 will be given an information handout on the Replication-First Hypothesis. Team 2 will be given an information handout on the Metabolism-First Hypothesis. The teams will have 30 minutes to understand their hypothesis. Students should take notes, as they will not be allowed to keep the original handout.

(2) Next, join back together in your 4-member working groups. The teams will educate each other on the different hypotheses. Each team will have 10 minutes to present and instruct the other team. At the end of this section, the entire working group should fully understand the differences between the Replication-First and Metabolism-First hypotheses. Listen carefully, ask questions, and take good notes.

(3) The teams will then separate into the 2-student teams again, but this time each team will be provided with a list of evidence that dismisses their initial hypothesis, as well as a list of evidence supporting the alternate hypothesis. In other words, Team 1, which was the team that presented information on the Replication-First Hypothesis, will be given information about experiments that support the Metabolism-First Hypothesis and undermine the Replication-First Hypothesis. Team 2, the team that presented information on the Metabolism-First Hypothesis, will be given information about experiments and facts that call the Metabolism-First Hypothesis into question and support the Replication-First Hypothesis. The teams have 30 minutes to review and understand the arguments in their handout. Taking notes is advised, as the argument handouts cannot be used in the next part of this debate.

(4) Each Team 1 must find and meet a Team 2 (it does not have to be the same as the original one they met in step 2). Each team has 10 minutes to present their evidence, followed by a 2-minute question period by the other team.

(5) Groups of four students (Team 1 *and* Team 2) choose the strongest arguments in favor of each hypothesis and against each hypothesis. Also, as a group, students decide which hypothesis is favored about the origins of life. Each team selects one member to present the group's decision to the class, rationalizing the decision.

Part II—Team 1 Information Handout: Replication-First (or Gene-First) Hypothesis

Life as we know it today consists of replication and metabolism. In our world, the DNA molecule's primary function is replication and proteins carry out a variety of chemical reactions required for metabolism. In contemplating which molecule arose first, a "chicken or the egg" problem arises. Let's assume that DNA was the first molecule of life. DNA can encode information, and its structure makes it easy to pass on this information to descendants. Unfortunately, DNA does not have catalytic ability and so cannot replicate on its own. It needs proteins. Next, let's assume that proteins arose first. Proteins are very versatile and can carry out a range of catalytic reactions. Unfortunately, they have no easy way of storing and passing on the information for making more of themselves. So, with proteins, we reach an impasse as well.

This dilemma was resolved by the proposal that RNA might have been the original life molecule. Unlike DNA, whose structure is constrained by a double-helix, RNA is singled-stranded and can fold in a variety of sequence-specific structures (see Figure 1). This structural variety is essential for the ability of a molecule to carry out a range of chemical reactions (the different shapes can confer the ability to catalyze different chemical reactions, like a protein enzyme). Since RNA is composed of building blocks that are similar to DNA, it shares DNA's ability to serve as an information molecule and its chemical structure offers a mechanism for replication (using base pairing). Thus, RNA may have been the first molecule of life because it has the potential to serve both as an information molecule and a catalytic molecule.

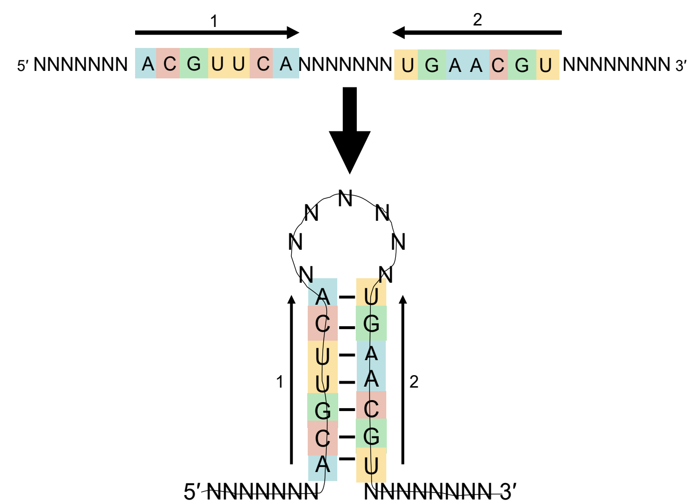


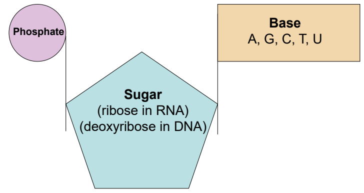
Figure 1. RNA Structure. RNA is a single-stranded nucleic acid molecule. Some of the nucleotides in its sequence can interact in a sequence-specific manner with other nucleotides located some ways away in the molecule. The example shown in this figure is a structure called a stem-loop (or hairpin), which forms when a stretch of nucleotides base pairs with a complementary stretch of nucleotides read in the opposite direction some ways away on the same molecule.

This idea was first suggested in 1968 by Carl Woese (Woese, 1968), and was given the name "RNA World Hypothesis" a few years later (Gilbert, 1986). The Replication-First or Gene-First Hypotheses are now nearly synonymous with the RNA World Hypothesis.

The RNA World Hypothesis

According to the RNA World Hypothesis, here are the proposed series of steps that led to the evolution of life on Earth (Joyce, 2002). The RNA World Hypothesis is dependent on the idea that organic molecules first accumulated on Earth. Among these molecules were the nucleobases (the purines adenine and guanine, and the pyrimidines uracil, thymine, and cytosine), and sugars (ribose and deoxyribose). Through chemical reactions, these chemicals assembled together to form ribonucleotides (a chemical composed of a ribose, a nucleobase, and a phosphate). In time, perhaps aided by mineral or clay catalysts, ribonucleotides assembled into long chain polymers to form RNAs of varying sequences (see Figure 2, next page).

These diverse RNAs accumulated on the planet, perhaps for millions of years. As each new RNA had a sequence of ribonucleotides that was randomly assembled, each RNA had a different sequence. Eventually, an RNA sequence was assembled that allowed the RNA to fold into a shape that gave it the catalytic ability to copy itself accurately. This replication reaction may have been very inefficient, perhaps taking as long as millions of years to occur. However, as soon as one molecule gained the ability to self-copy, its numbers increased among the pool of all RNAs. As each of the replicated RNA shared its parent's sequence, it also had the ability to replicate itself. There was an "explosion" in the number of RNA with the ability to replicate themselves. In time, the replicated RNAs came to dominate the pool of RNA on Earth.



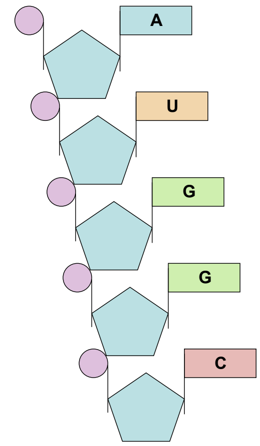


Figure 2. Building blocks of RNA. RNA is a polymer composed of a sequence of nucleotides. A single nucleotide is composed of 3 chemicals: a sugar (in RNA, the sugar is a D-ribose), a nucleobase (in RNA, the bases can be either adenine (A), cytosine (C), guanine (G), or uracil (U); in DNA, thymine (T) takes the place of uracil; other bases such as inosine exist but are not commonly found in living systems), and a phosphate group. For the RNA World Hypothesis to be possible, each of these chemicals should accumulate by abiotic processes on early Earth, and a chemical reaction must exist to create nucleotides from its building blocks. The above diagram represents the relative arrangements of these chemicals to form a nucleotide, but does not show every atom (i.e., the diagram is not a chemical structure). The diagram on the right shows the spatial arrangement of RNA by which a series of nucleotides are strung in a sequence. The sugars and phosphates serve as the scaffold to link the nucleotides together, while the bases are free to interact with other chemicals. A catalyst must have existed on early Earth to bring together and attach nucleotides to one another in this manner.

During this time, errors occurred when RNA copied itself. The changed sequences allowed the offspring to fold in a slightly different manner. Some errors allowed the RNA to replicate itself more quickly than its parent, perhaps by being able to bind more quickly to free floating ribonucleotides necessary for the chemical reactions. In replicating itself, it gave its offspring the ability to replicate faster. In time, RNAs of this sequence came to dominate the pool of RNAs on Earth. RNA molecules evolved in this manner for some time, becoming better, faster, and more efficient replicators in each generation.

How did life evolve from an RNA World to its current state? One idea is that certain sequences of ribonucleotides might have attracted and weakly bonded to specific amino acids that were accumulating on the planet. For example, perhaps the sequence of ribonucleotides in an RNA—Adenosine (A), Uridine (U), Guanosine (G)—attracted the amino acid methionine. The evolving RNA would serve as a template for protein synthesis by weakly attracting, binding, and holding in a specific orientation certain amino acids to the RNA. The amino acids would be held together long enough (and in the proper configuration) that the RNA could serve as a catalyst for the formation of a bond between the amino acids. If one RNA's sequence happened to favor the production of a protein that then either protected the RNA or helped it replicate, this RNA would be favored by natural selection and would prosper, leaving more descendants in the next generation. These RNAs would establish the early dynamics of gene expression as we know it (see Figure 3). In time, lipid membranes surrounded this primitive genetic system, protected the molecules from the environment, and ensured that the proteins produced by RNA did not diffuse away. This was the beginning of cellular life. Since DNA is more stable than RNA, it is better suited to store information and in time (through natural selection) replaced RNA. Similarly, proteins are much more versatile in the chemical reactions they can facilitate, and in time (through natural selection) took over the cell's catalytic functions and replaced RNA.

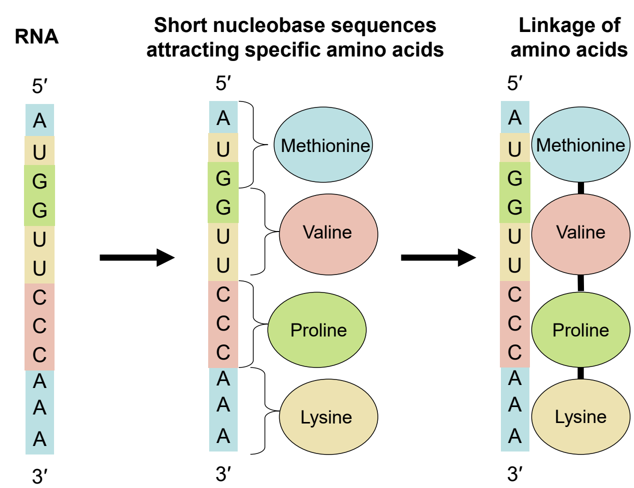


Figure 3. Evolution of early life from an RNA World to RNA and protein world. It is hypothesized that some sequences of bases attracted specific amino acids to the RNA. Holding several amino acids in such a close configuration for extended periods of time would favor their interaction and lead to the creation of a short peptide. Each RNA with identical sequence would produce similar peptides. If the peptide had an ability to protect the RNA or confer a catalytic advantage (e.g., by facilitating the creation of more RNA replicates), the RNA would gain the upper hand in replication and its sequence would take over the gene pool.

Part II—Team 2 Information Handout: Metabolism-First Hypothesis

Today, life has two essential properties: replication and metabolism. The scientific community has been mostly interested in the idea that replication evolved first. However, as our understanding of life increased, researchers began to contemplate the possibility that a series of self-sustaining chemical reactions (a chemical network) might be the ancestor of what we call life. After all, what is a cell but a series of orchestrated chemical reactions that extract energy from the environment to build order?

The Metabolism-First Hypothesis consists of several different hypotheses proposed by different researchers about how life first formed. These hypotheses are united by the idea that ordered chemical reactions, and not information replication, was the property of the initial life form. The interlocked networks of chemical reactions "evolved" in complexity over time. At some point in the evolution of the system, information molecules were incorporated into the system and life as we know it took form. The different hypotheses differ in the nature of the self-sustaining chemical reactions that characterized early life. One of these hypotheses will be described here, but many more exist.

Iron-Sulfur World Hypothesis

The Iron-Sulfur World Hypothesis was first proposed by Günter Wächtershäuser, a German patent lawyer (Wächtershäuser, 1988, 1990, 2000, 2006). The idea has garnered much recent attention in the scientific community. This idea proposes that mineral catalysts (such as iron-sulfide) present near deep sea hydrothermal vents are promoting a series of chemical reactions that could have promoted the evolution of life. The energy for the chemical reactions comes from the hydrothermal vents, specifically from the redox difference between the reduced briny hot water emerging from the mantle into the cold oxidized ocean.

This hypothesis depends on an understanding of some of the chemical processes that take place at deep sea hydrothermal vents. At these sites, hydrogen sulfide (H_2S) is expelled from the crust into the ocean. Hydrogen sulfide is a hydrogen-rich reducing gas so it can donate its electrons to other molecules. During this electron transfer, some energy is lost and can be used to drive other chemical reactions. Among other things, hydrogen sulfide can react with the mineral iron sulfide (FeS_2), known as pyrite or fool's gold. When hydrogen sulfide gives its electron to iron sulfide, the mineral transforms into the more reduced mineral troilite (FeS). Troilite can serve as a catalyst in many chemical reactions, giving off its electrons to organic molecules, and in the process reconverting itself to pyrite. More H_2S then comes out of the vents to convert pyrite into troilite, which then gives its electrons to organic molecules. So the vents are driving the chemical reactions (see Figure 4).

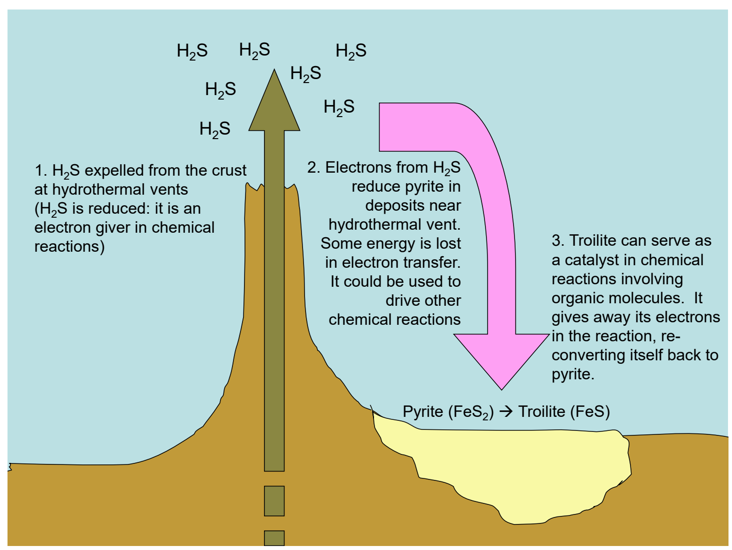


Figure 4. Chemical reactions occurring near hydrothermal vent systems. This environment is proposed to be the birthplace of life on Earth.

Wächtershäuser proposes the possibility that the surface of iron sulfide minerals catalyzes a series of chemical reactions that create a reverse Kreb cycle (or TCA or citric acid cycle) (see Figure 5, next page). The Kreb cycle is a series of chemical reactions that take place in all aerobic cells today. This cycle is key in extracting energy (electrons) out of reduced organic molecules such as sugars. At hydrothermal vents, the cycle is proposed to operate in reverse, taking in carbon monoxide or carbon dioxide and reducing it using the electrons provided from troilite to form more complex organic molecules that are abundant in many of today's life forms. The Kreb cycle in today's cells extracts energy out of reduced organic molecules, but the reverse Kreb cycle at hot vents produces reduced organic molecules. Among the molecules proposed to be produced are larger organic molecules such as acetate and pyruvate. In our cells, enzymes hold chemicals in the appropriate orientation such that they will react and in this manner catalyze the series of chemical reactions that transform one chemical into the next, ultimately stripping the electrons off of reduced organic molecules. In the reverse Kreb cycle, the iron-sulfur mineral takes the place of enzymes, binding chemicals in precise orientation to favor their reaction, adding electrons to small organic molecules. The role of today's enzymes is taken over by iron-sulfur minerals. Since this is a cycle, the end products of the chemical reactions are the reagents for the start of the next chemical reactions. In this way, the network is self-sustaining. This hypothesis also proposes how complex organic molecules could have built up in large quantity in a small area over time.

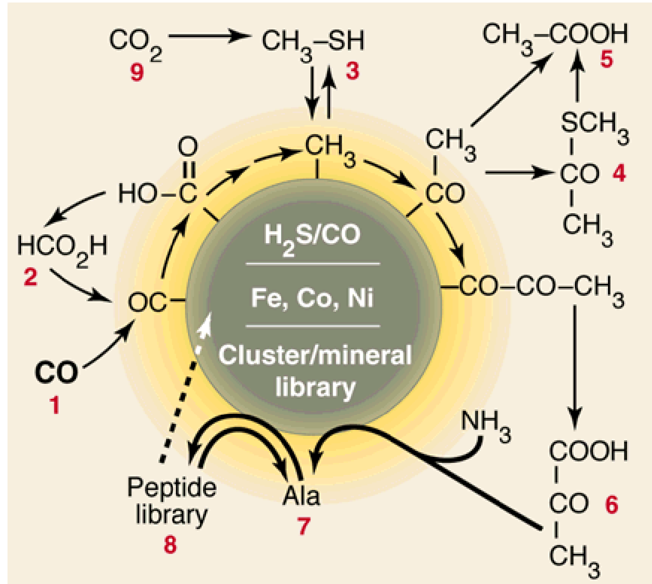


Figure 5. The reverse Kreb cycle proposed by Wächtershäuser to occur on the surface of iron-sulfur catalyst near hot vents. *Source:* Wächtershäuser, G. (2000). Origin of life. Life as we don't know it. *Science* 289(5483):1307–1308. Reprinted with permission from AAAS. This figure may be used for non-commercial and classroom purposes only. Any other uses require the prior written permission from AAAS.

Organic molecules will accumulate in the vicinity of the iron-sulfur catalyst. Some of them, particularly those with amphiphilic properties (e.g., some lipids), will aggregate, forming membranes. These membranes separate the iron-sulfur catalyst from the rest of the ocean, eventually forming a "cell" that encloses the "metabolic life form" (see Figure 6, Wächtershäuser, 1988, 2003, 2007). A slight variant on this idea comes from the observation that iron-sulfur often forms microscopic "bubbles" as it precipitates out of solution in the ocean (Russell & Hall, 1997). One group suggests that the first "cells" were encased not in a lipid membrane, but rather in an iron-sulfur casing (Martin & Russell, 2003; Lane, 2005). This permitted the compartmentalization of a primitive cell, provided the catalyst needed for chemical reactions to occur, and allowed for the accumulation of chemicals in a closed system. In time, as organic molecules accumulated in the cell, some of the lipids accumulated on the surface and caused the protocell to be enclosed within a membrane. This would give the "cells" the ability to travel away from their fixed origin in the metal bubbles (Martin & Russell, 2003). This departure from the site of origin may have occurred more than once, and could explain how different cell structures (bacteria, archaea) arose.

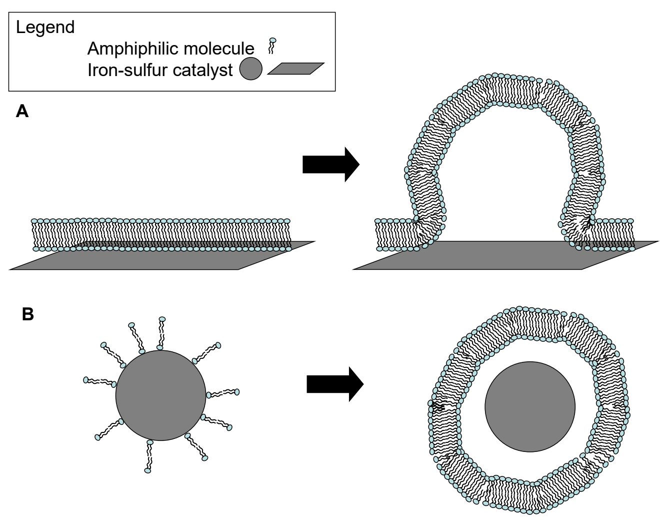


Figure 6. Two possible means (A and B) by which lipid-enclosed cells could have detached from their iron-sulfur catalyst. The mechanisms described here are the ones envisioned by Wächtershäuser and do not capture the "bubble" mechanism proposed by Martin & Russell. Figure drawn after ideas presented in Figure 9 of Wächtershäuser, G. (1988), "Before enzymes and templates: Theory of surface metabolism,"*Microbiological Reviews* 52(4):452–484.

Part III – Team 1: Intimate Debate Handout

Evidence for Metabolism-First Hypothesis (Iron-Sulfur World)

- In the laboratory, it is possible to use metals (nickel or nickel-iron precipitates) as catalysts to reduce carbon monoxide and create amino acids (Huber & Wächtershäuser, 1998, 2006).
- While amino acids are quickly degraded at hot temperatures (such as those found near hot vents), the presence of troilite (FeS) has been shown to substantially increase the stability of these molecules (Hazen et al., 2002). For example, the amino acid leucine has a half-life of less than 10 min at 200°C and pressure of 50 MPa. However, in the presence of troilite, the half-life of this amino acid increases to 30 hours.
- In the laboratory it is possible to activate amino acids and assemble them into peptides using either iron-sulfur or nickel-sulfur as a catalyst and carbon monoxide as a starting carbon source (Keller et al., 1994; Huber et al., 2003).
- A group designed a simulated hydrothermal vent system in the laboratory and reported the formation of elongated pores in the "vent system." The temperature gradient across the pore favored the accumulation of nucleotides at the bottom of the pore (Baaske et al., 2006). Nucleotides were concentrated up to 1,000 fold in the pores. A similar concentration in the pores that are formed near hydrothermal vents has also been observed for lipids (Budin et al., 2009). This concentration of nucleotides and lipids makes it more likely that such building blocks of life could react with one another to form more complex molecules, and solves the often cited problem of dilution of chemicals in the creation of life.
- Experiments reproducing the conditions of deep sea vents (hot temperatures, high pressure) explored the role of iron-sulfur as a catalyst in the creation of organic chemicals. It was found that simple molecules could be reacted on iron-sulfur to create more complex organic molecules such as pyruvate (a reduced organic molecule produced in our cells as a result of glucose break down and an intermediate in the production of many molecules such as amino acids—this is the chemical typically fed into the Kreb cycle in our cells) (Cody et al., 2000).
- In the laboratory, it is possible to use this system to show the creation of ammonium from nitrate, which is a potential precursor of nitrate reductase, an enzyme carrying out important biochemical reactions (Blochi et al., 1992).
- Three of the five reduction reactions in the reverse Kreb cycle have been shown to be possible in the presence of zinc-sulfur catalyst and UV light (Zhang & Martin, 2006) (see Figure 7).
- Several people have argued that the one process common to all life forms is a mechanism for generating a proton-motive force (a way of storing energy in the form of a proton gradient across a membrane—such as used by mitochondria, chloroplasts, and bacteria) (Koch & Schmidt, 1991; Lane, 2005). This mechanism of using energy is universal and must have been common in the earliest life form. The iron-sulfur catalyst could serve this function.
- Many enzymes in today's cells wrap around a metal core that serves as a catalyst in biochemical reactions (Johnson et al., 2005). An example is hemoglobin, whose iron core binds to oxygen as it is transported in the blood stream. Perhaps the reason so many proteins have an iron core is due to an ancestral role of iron in these chemical reactions.

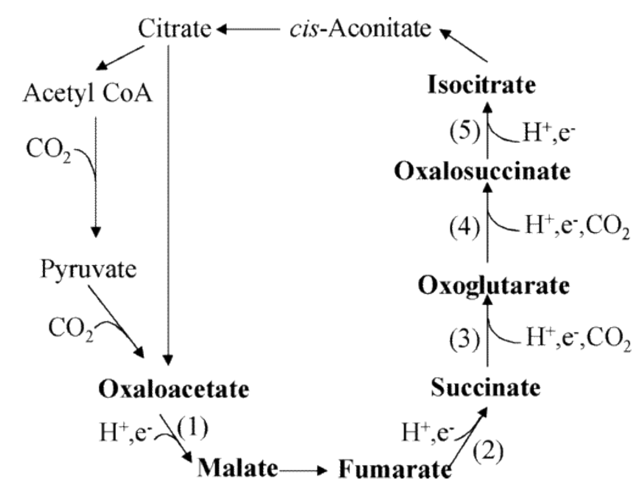


Figure 7. Reverse Kreb cycle, where the five reduction reactions are numbered and highlighted (bold). Reactions (1), (2) and (4) can be driven by a metal catalyst and UV light. Reprinted with permission from Zhang, X.V., and Martin, S.T., "Driving parts of Krebs cycle in reverse through mineral photochemistry," *J. Am. Chem. Soc.* 2006, 128 (50), pp 16032–16033. Copyright 2006 American Chemical Society.

- The Iron-Sulfur World Hypothesis assumes that the first cells were surrounded by metals, and that the synthesis of lipids permitted the escape of lipid-enclosed cells from their metal origin. Two escapes, separated in time, are proposed to explain the origins of bacteria and archaea. If this sequence of events is correct, then it would explain why bacteria and archaea have membranes composed of some entirely different types of lipids. Bacteria use fatty acid ester membranes, whereas archaea have isoprenoids in their membranes. If one assumes that archaea evolved from bacteria, then it is unclear how the ability to synthesize isoprenoids evolved. However, if one assumes two separate departures from a common metal cell, each characterized by the assembly of different lipid membranes surrounding the cell, then the lipid differences between the two cell types can be explained (see Figure 8) (Martin & Russell, 2003).
- Iron sulfide forms microscopic bubbles when it precipitates on the ocean floor. Some of these iron sulfate precipitates, with microscopic bubbles inside of them, have been found near hydrothermal vents that are 360 million years old (Martin & Russell, 2003). Such microscopic bubble structures are also formed in the laboratory by injecting a solution of chemicals representing hydrothermal fluids into a fluid representing the chemicals found in the primitive ocean (see Figure 9; Russell & Hall, 1997).
- Molecular phylogenies that use rRNA as a means to retrace the tree of life have found that the organisms at the root of the tree are thermophilic bacteria (bacteria that live in hot environments), as well as bacteria that thrive on sulfur, methane, or hydrogen, all chemicals likely to have been abundant at hot vents where putative biochemical networks first formed (Woese & Fox, 1977).

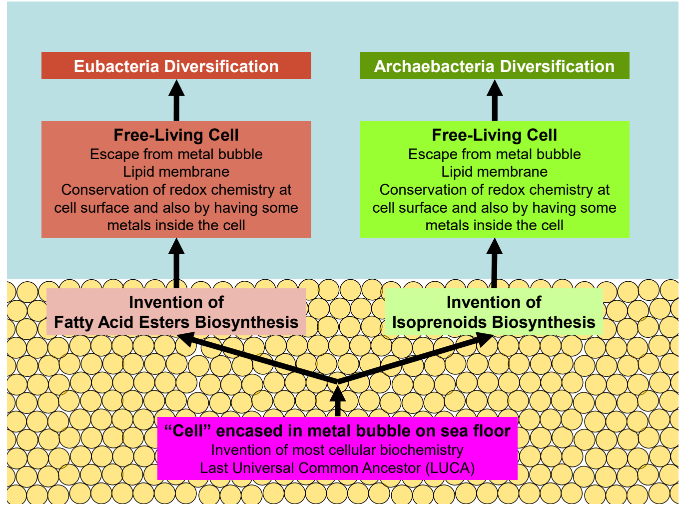


Figure 8. The iron-sulfur protocell model, combined with the hypothesis that at least two cells escaped the metal capsule by being enclosed in a lipid membrane, explains why the lipid membranes of bacteria and archaea differ in chemical composition. Figure drawn after Figure 5 of: Martin, W., and M.J. Russell (2003). On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Philos Trans R Soc Lond B Biol Sci.* 358(1429):59–85.

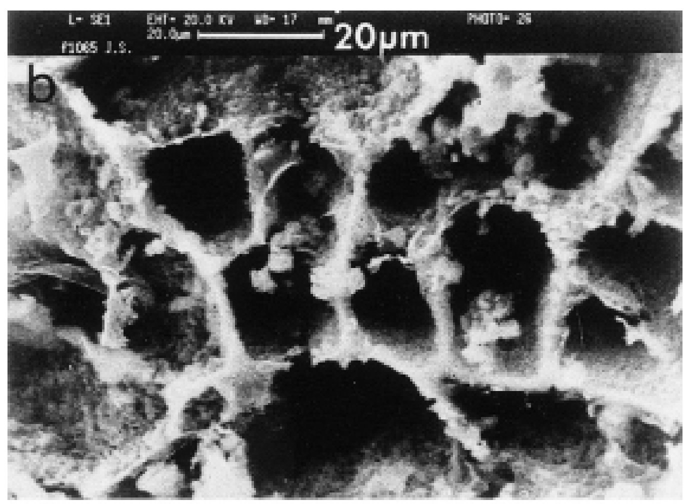


Figure 9. Microscopic bubbles formed by injecting Na_2S solution (representing hydrothermal fluid) into $FeCl_2$ solution (representing the iron-bearing Hadean ocean). Photograph by John Sherringham, from Figure 2 of: Russell, M.J., and Hall, A.J. (1997). The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *J. Geol. Soc. Lond.* 154: 377–402. Use of item in accordance with GSL guidelines (http://www.geolsoc.org.uk/gsl/publications/page417.html).

Evidence against Replication-First Hypothesis

If the Replication-First Hypothesis is true, then nucleotides (and its building blocks: the nucleobases, ribose and phosphates) must be formed by chemical reactions that took place under the conditions of early Earth. However…

- Most of the chemical reactions shown to produce organic molecules depend on the absence of oxygen in the atmosphere (a reducing environment). However, whether there was oxygen in the early Earth's atmosphere is the subject of some debate (Kasting, 1993). Furthermore, experiments have shown how difficult it is to produce the building blocks of life (the nucleobases [purines and pyrimidines], sugars, amino acids) when oxygen is present (Stribling & Miller, 1987).
- Some nucleobases are found in meteorites. However, there is the possibility that the meteorites have been contaminated by terrestrial sources of nucleobases, or that contamination occurred during the analysis of the sample (Van der Velden & Schwartz, 1977).
- While the nucleobases adenine and guanine are fairly easy to generate by chemical reactions that are proposed to have taken place on early Earth, the synthesis of the nucleobases cytosine and uracil (which are also required for the formation of RNA) are more problematic. Some chemical reactions have been proposed, but depend on specific environmental conditions that are unlikely to have existed in large enough areas to explain the prevalence of cytosine and uracil in the genetic code (Robertson & Miller, 1995).
- Many chemical reactions proposed to explain the synthesis of organic molecules require a high temperature. At 100°C, nucleobases are destroyed easily. The half-life of adenine and guanine is one year, uracil is 12 years, and cytosine is 19 days (Levy & Miller, 1998). This instability makes it unlikely that nucleobases could have accumulated in sufficient quantities on the early Earth. Note also that an impact from an asteroid (a proposed delivery vehicle) would likely boil the oceans and destroy any of these compounds that had built up in the oceans.
- It has been shown experimentally that ribose can be synthesized by the formose reaction using formaldehyde as a building block. However, the yield is very small, the sugars produced are unstable, degrading very rapidly, and the reaction does not take place in the presence of nitrogenous substances (which are needed for the synthesis of nucleobases) (Shapiro, 1988). Thus, even if ribose was formed, it would be under conditions that are such that it would be unlikely to then form nucleotides.
- Ribose can exist in two mirror image forms: D- and L-ribose. In RNA today, all ribose is D-ribose. In a replication reaction where the RNA template is composed of D-ribose, if a mixture of nucleotides composed of D- and L-ribose are available for the replication, the incorporation of an L-ribose blocks the further elongation of the chain (it is a chain terminator) (Joyce et al., 1984). This poses a problem for the emergence of life, if nucleotides of D- and L-ribose were available in the prebiotic soup. Unless the ribozyme that replicated the chain could discriminate against L-ribose, most replicated chains would be aborted.
- The chemical reaction that is known to form ribose also forms a vast number of other sugars (Larralde et al., 1995). How could ribose have accumulated in large enough quantity and been selected for inclusion in nucleotides?
- Early Earth conditions lent themselves to the creation of many molecules that are structurally and functionally similar to the ones that were ultimately incorporated in the building blocks of life (e.g., the purines adenine and guanine are not the only purines that exist: there is also xanthine and isoguanine). Given this prebiotic "clutter," why and how were only certain chemicals selected and others excluded? This is currently not appropriately explained by the RNA World hypothesis (see Figure 10, next page).
- A nucleotide is made of a nucleobase, linked to ribose, linked to phosphate. However, the phosphate-ribose bond, which is crucial to the formation of nucleic acids, is prone to hydrolysis and is therefore unstable (Lindahl, 1993). How could this building block have accumulated in large enough quantities under these conditions?

If the replication-first model is true, then nucleotides must have assembled with others in the correct 5′ to 3′ linkages that create RNA and DNA. However…

- The formation of RNA requires that all nucleotides be attached using 3′-5′ phosphodiester bonds on their ribose. While several proposed conditions for the polymerization of nucleotides have been proposed, all allow the possibility that some of the links between nucleotides will be 2′-5′ bonds (Orgel, 2004). This would create unstable polymers.

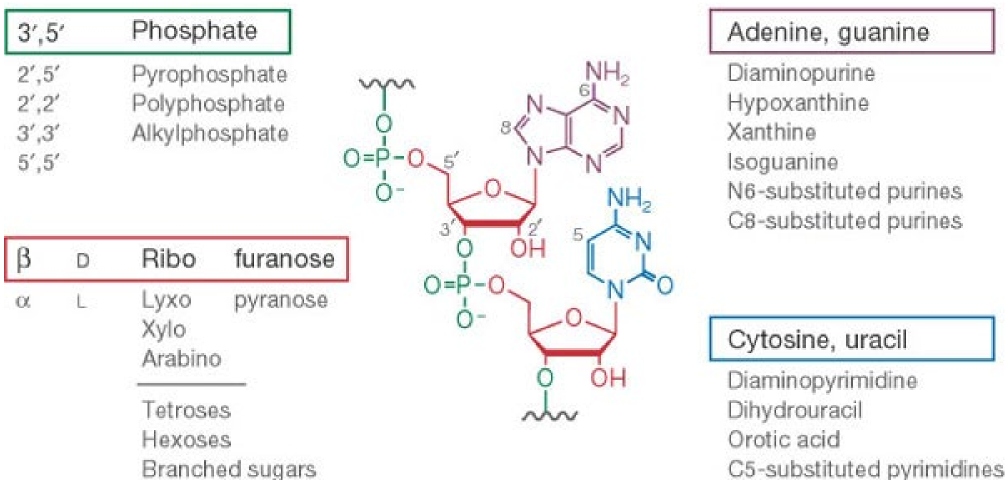


Figure 10. Building blocks of RNA, and the similar chemicals present on early Earth. Each of the building blocks of RNA are boxed. Below each one are written some structurally similar molecules that may have co-existed with the RNA building blocks at the time of the origins of life. Any of the alternative chemicals could have been used in the formation of RNA. Why were some molecules selected and others excluded from the building blocks of life? *Source:* Figure 2 from Joyce, G.F. (2002). The Antiquity of RNA-based evolution. *Nature* 418: 214–221. Reprinted by permission from Macmillan Publishers Ltd: *Nature*, copyright 2002.

- RNA is an unstable molecule. In alkali solution, the 2′OH group of ribose makes a nucleophilic attack on the phosphate group linked to the 3′ carbon. The net effect is that the linkages between nucleotides are broken, and the RNA molecule is degraded to its constituent nucleotides (see Figure 11). In an RNA World, an RNA molecule would be made and broken-down in a short time. If the ribozyme does not function very effectively, the RNA molecule will be degraded before it has a chance to make a copy of itself.
- Some ribozymes capable of replicating RNA have been created in the laboratory by artificial selection. However, these ribozymes are not very accurate, and often incorporate the wrong nucleotide during the copying process. It has been calculated that the error rate of the best ribozyme isolated thus far is about five times higher than would be needed for an RNA of that size to be able to faithfully replicate itself (Bartel, 1999).
- A recent report of a ribozyme capable of replicating a part of itself could only copy 20 nucleotides out of 200 (Zaher & Unrau, 2007).
- When ribozymes copy an RNA using base-pair rules, the newly replicated RNAs are complementary in sequence to the starting RNA (e.g., if the original RNA was 5′AUG3′, the replicated RNA

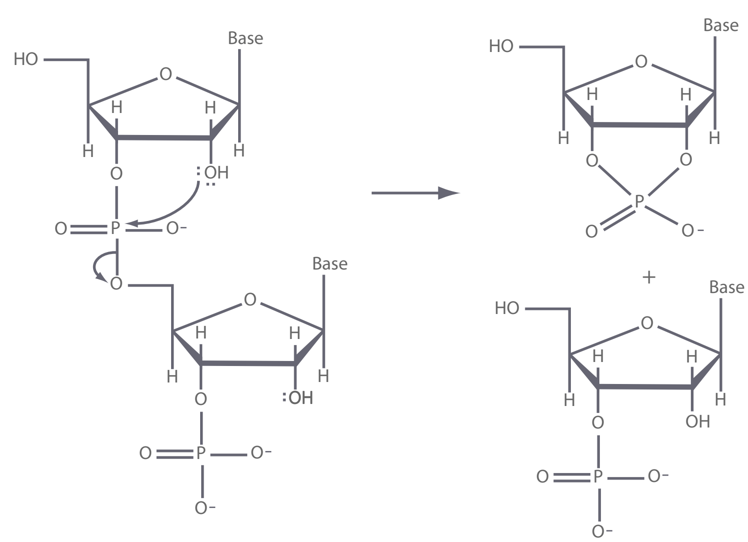


Figure 11. RNA instability caused by the propensity of RNA to cleave itself. The ribose's 2′ OH is shown to attack the 3′ linkage. This reaction is facilitated by incubation in an alkali solution.

is 5′CAU3′). Since the sequence of the RNA is different, the copy would not preserve the catalytic function of the original molecule. This poses a problem in terms of explaining how the sequence of the RNA with catalytic activity increased in the RNA pool (in other words, it cannot be selected).

Other Problems…

- Many enzymes today require a nucleotide cofactor. These cofactors are argued to be the remnant of the RNA World. In other words, in the past, RNAs carried out the reactions that enzymes carry out today. For this to be true, in the RNA World, RNAs would have had the ability to carry out oxidation-reduction reactions and reactions involving carbon-carbon bonds (beyond Diels-Alder reactions). To date, no ribozyme (naturally occurring or evolved in the laboratory) has been identified that can carry out these types of chemical reactions (Bartel & Unrau, 1999).

Part III – Team 2: Intimate Debate Handout

Evidence for Replication-First Hypothesis

If the Replication-First Hypothesis is true, then nucleotides must be formed by chemical reactions that took place under the conditions of early Earth. Remember that a nucleotide is made up of nucleobase, a sugar (ribose, deoxyribose), and a phosphate.

- Adenine, one of the nucleobases in RNA and DNA, can be synthesized by simple chemical reactions in which hydrogen cyanide and ammonia (gases believed to be present on the early Earth) are reacted (Oró, 1961).
- The clay montmorillonite, which is created by the weathering of volcanic ash and is believed to have formed large deposits on early Earth, seems to facilitate chemical reactions that lead to the formation of the nucleobases adenine, cytosine, and uracil (Saladino et al., 2004; Ferris, 2005). In addition, it seems to stabilize the nucleobases adenine and guanine while enhancing the degradation of thymine (which is not found in RNA, and whose absence must be accounted for).
- Adenine could have formed on Earth or could have formed in outer space by the above chemical reaction. Glasner et al. (2007) have shown that under conditions found in outer space (where hydrogen cyanide is common), adenine can form.
- Adenine is one of the chemicals found in the cometary dust particles surrounding Halley's Comet (Kissel & Krueger, 1987).
- The nucleobases uracil and xanthine[1] are found in the Murchison meteorite that fell in Australia in 1969 (Martins et al., 2008). Similarly, the nucleobases adenine and guanine were identified in a sample from the Orgueil meteorite (Hayatsu, 1964). Guanine, and possibly xanthine and hypoxanthine were identified in the Antarctic meteorites Yamato (Y-) 74662 and Y-791198 (Shimoyama et al, 1990).
- Some people have argued that the nucleobases found in meteorites are due to terrestrial contamination. However, the nucleobases found in these meteorites occur in a proportion that does not match their proportion on Earth (Van der Velden & Schwartz, 1977). Also, the proportion of isotopes of hydrogen, carbon, and nitrogen found in these organic molecules do not match those found on Earth (Yuen et al., 1984).
- Ribose can be formed under certain conditions by chemical reactions involving formaldehyde as a starting point (the formose reaction) (Shapiro, 1988).
- The formation of ribonucleotides (nucleobase, ribose, phosphate) was a mystery for a long time. There were no known reactions that would attach a base to a ribose under conditions supposed to have existed on the early Earth. A solution to this dilemma was recently published. Using conditions that are plausible for the early Earth, nucleotides were created. However, these nucleotides were synthesized not from ribose and nucleobases, but from other chemicals such as arabinose amino-oxazoline and anhydronucleoside intermediates. In other words, the key lay not in synthesizing each building block separately and assembling them to build a nucleotide, but rather in assembling the final product in a completely different manner from scratch, never passing by the building block stage. The chemical reactions used to create nucleotides used a completely different set of intermediates (Powner et al., 2009).
- Many organic molecules can exist in mirror image forms. This is true of ribose, which can exist in D form, or its mirror image L. In RNA, only the D-ribose is used. While the reason that D-ribose was exclusively selected remains uncertain, theoretical calculations and recent experiments have suggested that the D-ribose is the more stable form (Bolik et al., 2007). Natural selection would explain why D-ribose is the form used in living cells today.

If the RNA World Hypothesis is correct, then nucleotides need to assemble into short RNA or DNA polymers.

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[1] Xanthine is not found in DNA and RNA, but is an important intermediate in the synthesis of adenine and guanine.

- Montmorillonite clay can serve as a catalyst to favor the creation, and extension of nucleotide chains that are 30-50 nucleotides long (Ferris, 1996, 2002, 2005; Huang & Ferris, 2003) (see Figure 12).
- Lohrmann & Orgel showed that nucleotides could be polymerized without the help of enzymes using activated nucleotides such as nucleoside 5′phosphorimidazolides (Lohrmann & Orgel, 1976). This resulted in RNA chain lengths of up to 15 nucleotides. (Note that while this demonstrated the feasibility of synthesizing RNA from its building blocks, nucleoside 5′phosphorimidazolides are not believed to be formed under prebiotic conditions.)
- Frozen sea ice has properties which favor the elongation of RNA, using imidazole, a template, but no enzymes. This is like the Orgel reaction, but longer chain lengths (of up to 420 nucleotides) were obtained (Trinks et al., 2005).
- In the presence of lipids, heat, and conditions that fluctuate between hydration and dehydration, it has been found that nucleotides assemble to form chains of up to 100 nucleotides long (Rajamani et al., 2008). These nucleotides are surrounded by lipid membranes, providing a protective environment for the RNA and suggesting how a cell might have formed. The conditions necessary for these reactions can be found near hot springs.

If the RNA World Hypothesis is correct, then RNAs must have, in addition to their coding ability, some capacity to carry out chemical reactions.

- Single nucleotides have recently been shown to have catalytic activity (Kumar et al., 2010).
- RNA sequences with catalytic activity have been found in the cell. These are called ribozymes (Dudna & Cech, 2002) (see Figure 13, next page). These RNAs have enzyme-like capabilities, and can carry out chemical reactions in the absence of proteins. The first reported ribozyme was the 26S rRNA of *Tetrahymena thermophila*. This rRNA was shown to have the ability to splice out its own intron without the help of any protein (Kruger et al., 1982). This discovery was followed by many more findings of RNA sequences that have essential catalytic functions in the cell.

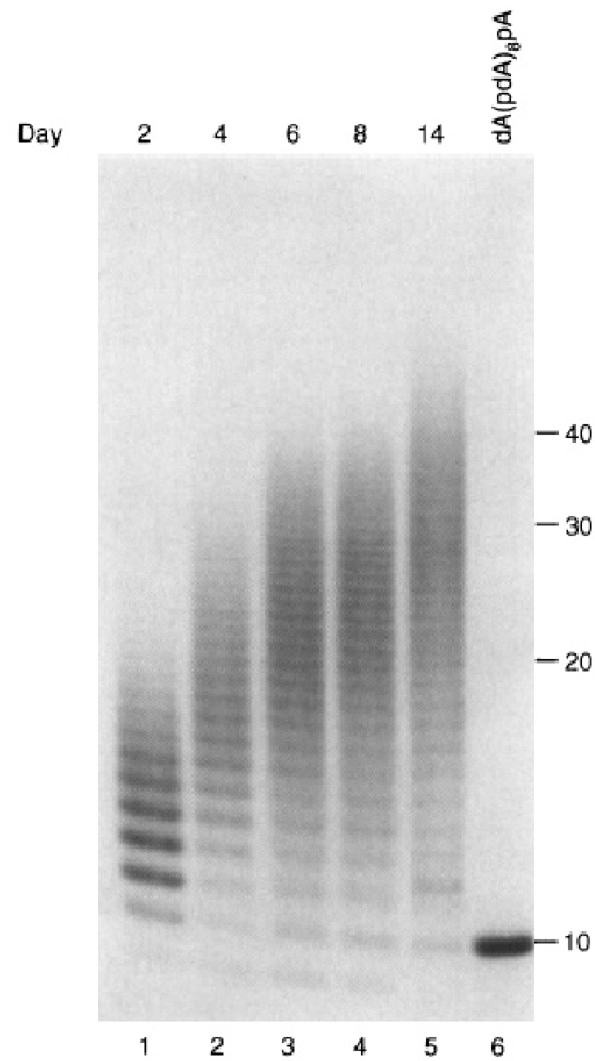

Figure 12. Nucleotides polymerize to form chains of 30-50 in the presence of montmorillonite clay. In this experiment, activated deoxynucleotides (building blocks of DNA) and a 10-nucleotide-long DNA molecule were incubated for several days with montmorillonite clay. The products were extracted from the reaction on the days indicated above the graph, and the products obtained were separated by size. The dark smears are the DNA products obtained. The "higher" they are on the picture, the longer the chains (the numbers on the right represent the number of nucleotides in the chain). The experimenters report that no elongated products were obtained in the absence of montmorillonite (data not shown).

Source: Figure 2 from Ferris, J.P., Hill, A.R. Jr,. Liu, R., and Orgel, L.E. (1996). Synthesis of long prebiotic oligomers on mineral surfaces. *Nature* 381(6577): 59–61. Reprinted by permission from Macmillan Publishers Ltd: *Nature*, copyright 1996.

- RNAs have been confirmed to have a range of catalytic activity (Joyce, 1998). In today's biological systems, RNAs are known to catalyze phosphoester transfer reactions, phosphoester hydrolysis, and peptide bond formation. *In* *vitro,* RNA molecules can be made to help in nucleotide ligation, nucleotide phosphorylation, aminoacyl transfers, amide bond formation or cleavage, peptide bond formation, to list a few.

- Some putative descendants (remnants) from these early catalytic RNA life forms exist in our cells. Ribosomes, the cellular machines composed of RNA and protein, play an essential role in protein assembly. The RNA part of ribosomes carries out the chemical reaction of assembling amino acids to form the protein. Similarly, spliceosomes, cellular machines composed of RNA and proteins, remove introns from mRNAs. The RNA component of the spliceosome plays a crucial role in carrying out this chemical reaction. Other examples include ribonuclease P, and hammerhead ribozymes. Also, nucleotides act as co-factors for many enzymes (e.g., NAD^+, FAD, coenzyme A) (White, 1976). ATP, another ribonucleotide, is used by the cell to transfer energy between chemical reactions. Finally, microRNAs have a range of regulatory roles in the cell.

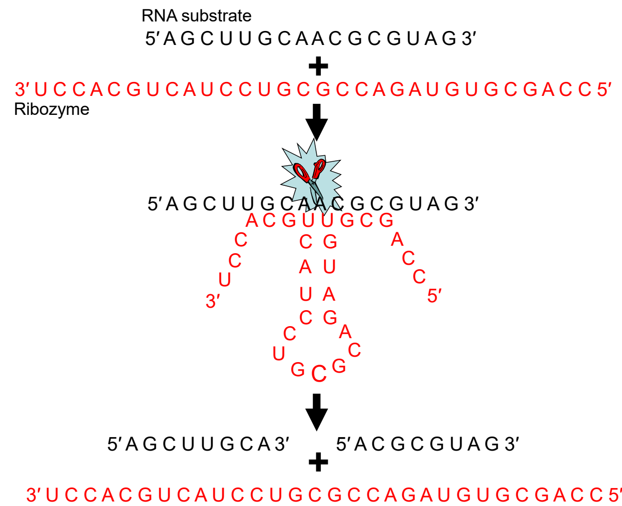

Figure 13. Ribozymes are RNA molecules with catalytic activity. Each ribozyme is capable of a different type of chemical reaction. Portrayed here is a hypothetical enzyme catalyzing the cleavage of a specific RNA between specific nucleotides. By virtue of the ribozyme's sequence and complementary base pairing, it is able to recognize a specific RNA molecule (its substrate). The ribozyme interacts chemically with the substrate molecule to weaken the chemical bond between two specific nucleotides in the target's sequence (the chemistry is not shown but is symbolized by the scissors). The ribozyme then releases the two products and is itself left unaltered, to repeat this catalysis on another molecule.

- In the laboratory, RNAs underwent several rounds of artificial selection for their ability to synthesize pyrimidine nucleotides (Unrau & Bartel, 1998). RNA molecules capable of catalyzing this chemical reaction were identified, suggesting a possible way in which RNAs could have contributed to the formation of more of their building blocks to sustain their existence.
- As the RNA World transformed into our current world, one of the required steps in the evolution is the ability of RNAs to transfer specific amino acids onto specific RNA sequences (in other words, an aminoacyl-tRNA synthetase ribozyme). In the laboratory, RNAs underwent several rounds of artificial selection for their ability to carry out this reaction. RNAs capable of transferring a glutamine amino acid onto a target tRNA was achieved (Lee et al., 2000).
- As the RNA World evolved into our current world, one of the required steps in the evolution is the ability of RNAs to link together individual amino acids into protein. This step is today carried out by the ribosome, but initially would have to have been carried out by RNA alone. In the laboratory, RNAs underwent several rounds of artificial selection and an RNA sequence capable of accomplishing this chemical reaction was identified (Zhang & Cech, 1997).

Can RNA copy itself (and evolve)?

- In the laboratory, RNAs underwent several rounds of artificial selection for their ability to copy another RNA template (Johnston et al., 2001). An RNA capable of using an RNA template and extending a primer by 14 nucleotides was identified. This suggests that RNAs can have the catalytic ability to replicate accurately.
- A ribozyme was artificially selected in the laboratory for its ability to replicate itself (Zaher & Unrau, 2007). This 200 nucleotide ribozyme could copy a 20 nucleotide section of itself.
- Recently, an RNA enzyme was created that links specific oligonucleotides together. Using two such enzymes and four different oligonucleotide substrates, the RNAs were able to catalyze each other's synthesis. Thus, the RNAs

were able to replicate themselves faithfully. This replication capability occurred in the absence of proteins or other biological materials (Lincoln & Joyce, 2009).

Other supporting evidence

- One of the problems that has been cited against the RNA World is that once encased inside a protocell, there would be no more supply of nucleotides to replicate an RNA molecule (nucleotides cannot penetrate a lipid bilayer). A possible way around this has been the proposal that protocell membranes were composed of fatty acids, their corresponding alcohols, and glycerol monoesters. Membranes composed of such chemicals allow the passage of charged molecules such as nucleotides (Mansy et al., 2008).
- RNA is a very unstable molecule. The clay montmorillonite, which is created by the breakdown of volcanic ash and is believed to have been abundant on the early earth (Ferris, 2005), has been shown to help protect ribozyme against the degradation effects of UV light (Biondi et al., 2007).

Evidence against Metabolism-First Hypothesis (Iron-Sulfur World)

- In the laboratory, there is evidence that iron-sulfur can serve as a catalyst to drive chemical reactions that transform carbon monoxide to more complex organic molecules. However, the evidence also suggests that in the presence of carbon dioxide (as would be present in the ocean), these reactions do not take place (Keefe et al., 1995). This shows that the proposed system lacks robustness and that it would be unlikely to explain the origins of metabolism or of life.
- Simulations have investigated the rate at which iron-sulfur works as a catalyst to promote chemical reactions that first created organic molecules, such as amino acids and then polymers (of 20-mer) of these compounds (Ross, 2008). These studies suggest that this mechanism does not operate quickly enough to account for the emergence of life in the one billion years between the end of the heavy bombardment period and the appearance of cellular life.
- The ease with which troilite gives its electrons to carbon has been studied as a function of temperature. It was found that as the temperature increases (as would be expected near a hot vent), the ease with which the electron transfer takes place decreases (Schoonen et al., 1999). Thus, it seems unlikely that iron-sulfur can serve as a catalyst in the biochemical reactions proposed by Wächtershäuser.
- Iron-sulfur, serving as a catalyst in the formation of early biochemicals, cannot explain why only one form (one mirror image) version of amino acids (L-amino acids) were included in proteins, and one type of sugar (D-ribose) is included in nucleotides and their polymers (RNA and DNA) (Martin & Russell, 2003).
- One critical question remains to be answered: without any genetic or informational material, how could metabolic life forms reproduce or evolve?
- Overall this hypothesis has not been subjected to much experimental scrutiny.

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