The Molecular Origins of Life: Replication or Metabolism-First? Introductory 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?
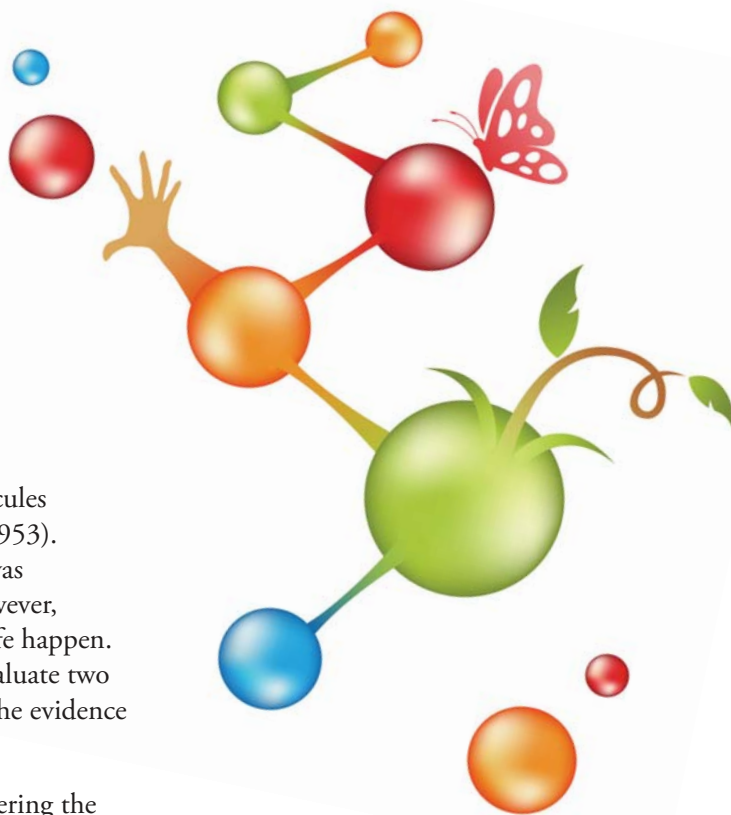
Part II – Gaining Knowledge of One Hypothesis: Jigsaw Instructions

(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.

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.

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.

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.
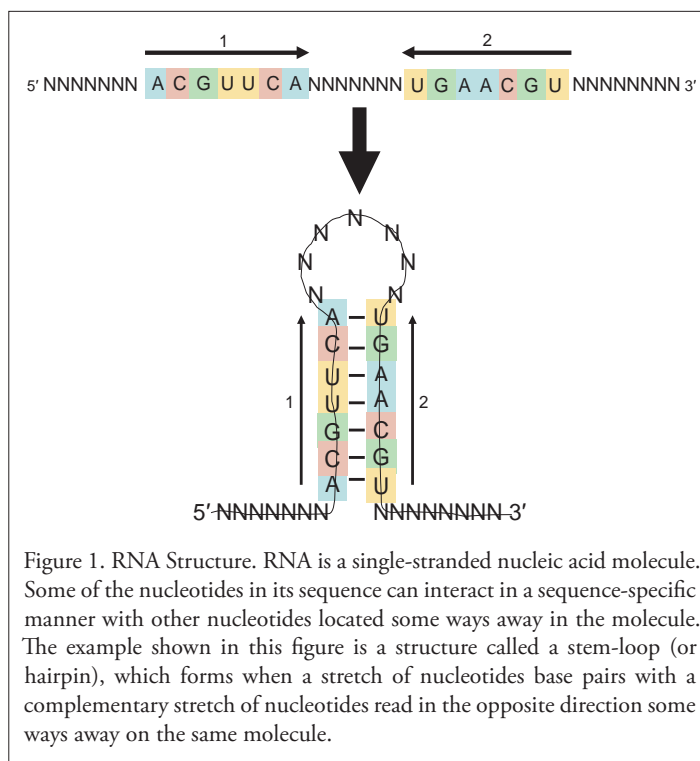
Figure 2. Building blocks of RNA. RNA is a polymer composed of a sequence of nucleotides. A single nucleotide is composed of 3 chemicals: a sugar (in RNA, the sugar is a D-ribose), a nucleobase (in RNA, the bases can be either adenine (A), cytosine (C), guanine (G), or uracil (U); in DNA, thymine (T) 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.
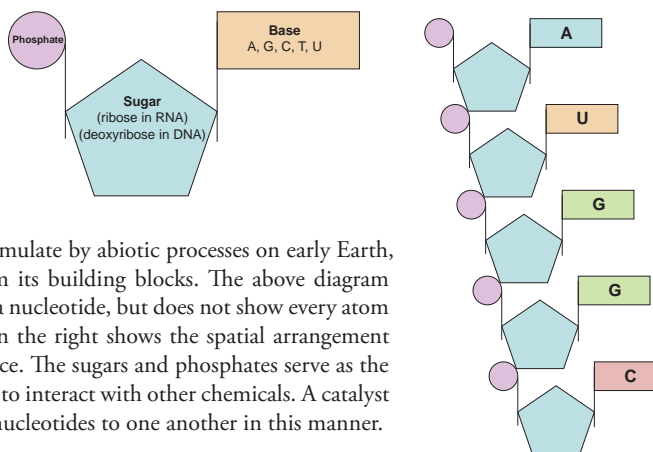
During this time, errors occurred when RNA copied itself. The changed sequences allowed the offspring to fold in a slightly different manner. Some errors allowed the RNA to replicate itself more quickly than its parent, perhaps by being able to bind more quickly to free floating ribonucleotides necessary 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 hold-ing 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 for-mation 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 expres-sion as we know it (see Figure 3). In time, lipid membranes surrounded this primitive genetic system, protected the molecules from the environ-ment, 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.
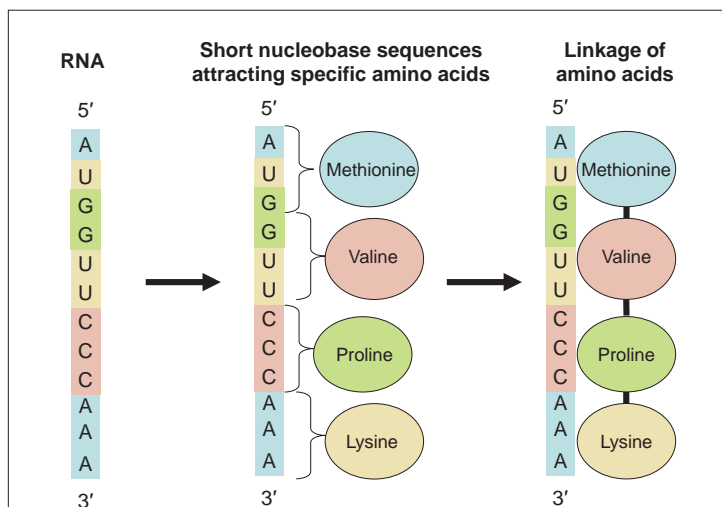
Figure 3. Evolution of early life from an RNA World to RNA and protein world. It is hypothesized that some sequences of bases attracted specific amino acids to the RNA. Holding several amino acids in such a close configuration for extended periods of time would favor their interaction and lead to the 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.

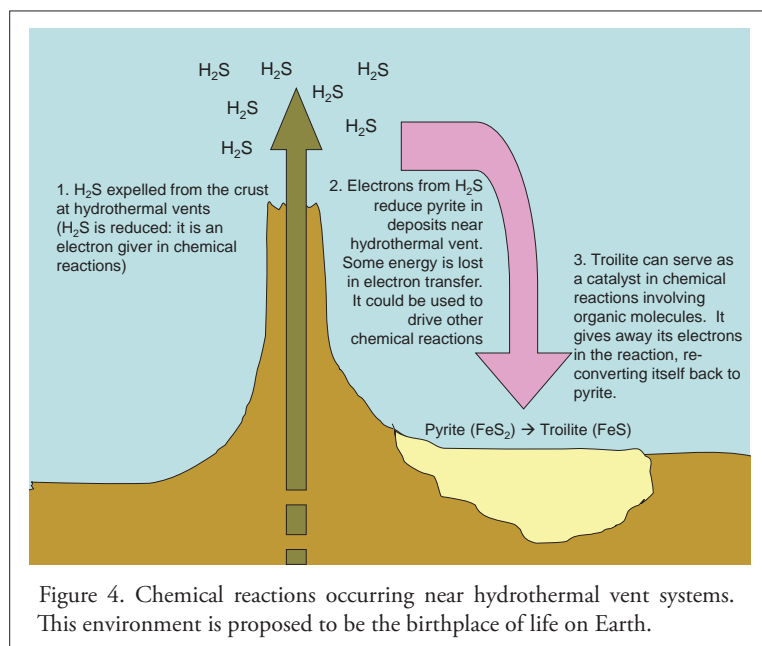# Part II—Team 2 Information Handout: Metabolism-First Hypothesis

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.
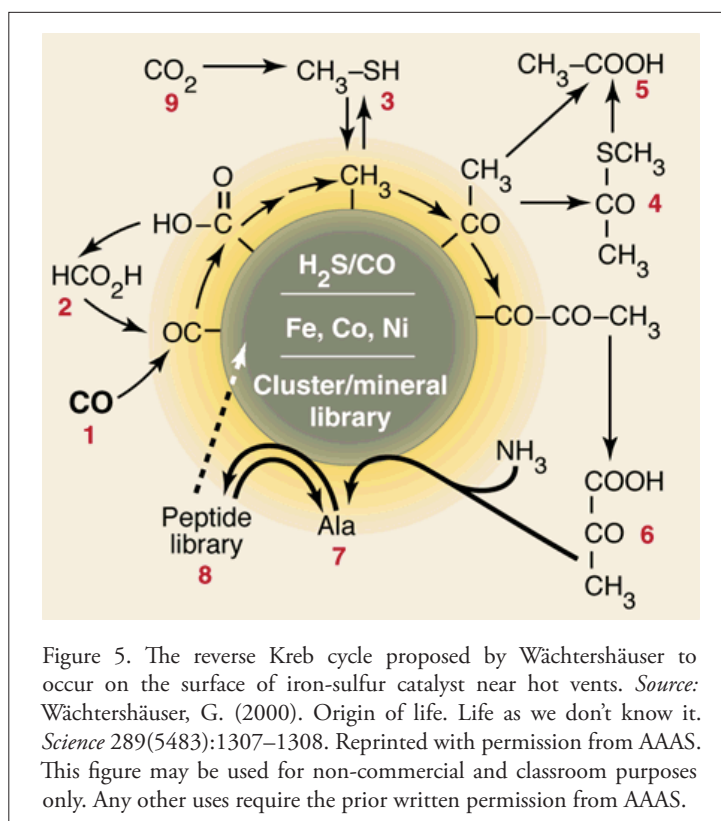
This hypothesis depends on an understanding of some of the chemical processes that take place at deep sea hydrothermal vents. At these sites, hydrogen sulfide (H_2S) is expelled from the crust into the ocean. Hydrogen sulfide is a hydrogen-rich reducing gas so it can donate its electrons to other molecules. 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.
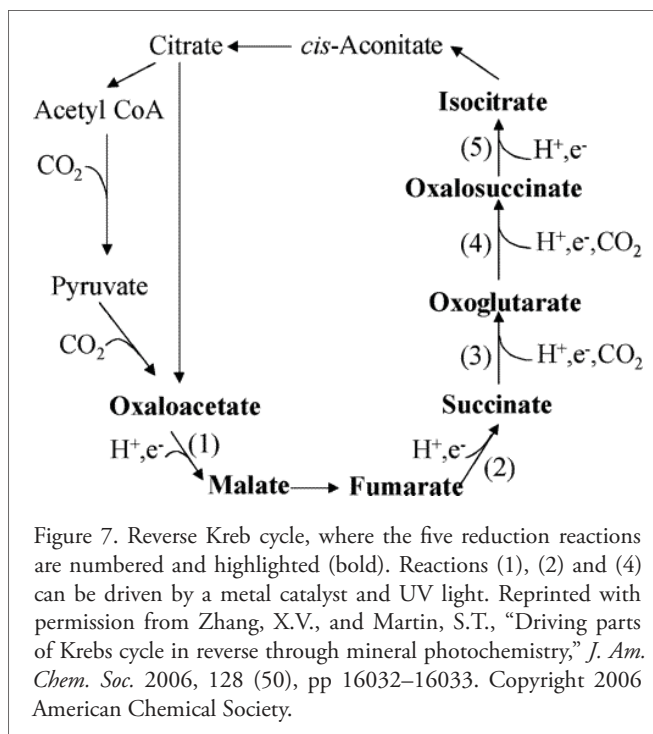
Organic molecules will accumulate in the vicinity of the iron-sulfur catalyst. Some of them, particularly those with amphiphilic properties (e.g., some lipids), will aggregate, forming membranes. These membranes separate the iron-sulfur catalyst from the rest of the ocean, eventually forming a "cell" 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)

Many Metabolism-First Hypotheses, including the Iron-Sulfur World Hypothesis, started from theoretical musings. But, is there any experimental evidence to support the Iron-World Hypothesis? Let's first look at the possibility that the proposed chemical reactions can take place in the presence of a metal catalyst. One research team reports that nickel can be used to create more complex and reduced organic molecules (such as amino acids) from simple ones such as carbon monoxide. Another experiment that mimicked hydrothermal vent conditions and utilized iron-sulfur as a catalyst produced pyruvate from simple molecules. Pyruvate is a reduced organic molecule produced in our cells as a result of glucose break-down, as well as an intermediate in the production of many compounds such as amino acids. Notably, it is the molecule that our cells feed into the Kreb cycle (a series of chemical reactions used to extract energy from chemicals). Finally, another group of experimenters has shown that three of the five reduction reactions in a reverse Kreb cycle can occur in the presence of a zinc-sulfur catalyst and UV light (see Figure 7).

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.

If early life used metal catalysts, could there be remnants of this ancestral lifestyle in our cells? Indeed, many essential proteins today require the presence of a metal atom in their structure to carry out the catalytic function. This is in part why minerals (iron, zinc, magnesium, etc.) are essential in our diet. As an example, hemoglobin, the protein that carries oxygen in the bloodstream from lungs to tissues, holds four iron atoms at its core, and it is these atoms that bind to and carry oxygen.

What about the hydrothermal vent environment? First, laboratory simulations of hydrothermal vents have found that small pores form in the vent system. In these pores, the temperature is suitable for chemical reactions (cooler than near the vent itself, which is too hot for the stable formation of chemicals), and furthermore that the pores serve as receptacles to concentrate the organic molecules that form, favoring further interactions. In a separate observation, iron-sulfide is known to form microscopic bubbles when it precipitates on the ocean floor near hydrothermal vents. The Iron-Sulfur World Hypothesis proposes that the first cells were enclosed in a metal casing, which would afford protection, serve as a catalyst for biochemical reactions, concentrate the produced chemicals, and prevent their diffusion. The discovery of the iron-sulfide bubbles provides a possible environment where such a system could have occurred. 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. Supporting this hypothesis is the observation that bacteria and archaea have membranes composed of 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 (Figure 8, next page).

Finally, phylogenetic analysis of all living organisms has determined that thermophilic (heat-loving) archaea and bacteria that thrive on sulfur, methane and hydrogen are at the root of the evolutionary tree. All of these conditions are found

in hydrothermal vents, supporting the emergence of life from that environment.

Evidence against Replication-First Hypothesis

What is some of the evidence that argues against the RNA World Hypothesis?

First, while the RNA molecule is composed of a select set of building blocks, many other closely-related analogs were probably present during the formation of life. Why did some variants get included in life and others get excluded? For example, there are more than just four nucleobases that can be created by chemical reactions, yet only adenine, guanine, cytosine, and uracil were included in the structure of RNA. The RNA World Hypothesis has a difficult time explaining how RNAs formed using consistent building blocks in the presence of so much chemical clutter.

Second, can nucleotides be induced to link together using consistent linkages to form a stable RNA? There are several ways in which nucleotides can be assembled to form a strand of RNA (Figure 9). RNA is typically assembled by forming a bond between a specific carbon on the ribose of one nucleotide, a phosphate, and a specific carbon on the ribose of the adjacent nucleotide. However, it is chemically possible to involve other ribose carbons in the formation of the bond. Indeed, many of the chemical reactions that create RNA in the laboratory (under conditions mimicking early Earth) produce RNA molecules that link nucleotides in different manners. In living systems, protein enzymes ensure that all the bonds are of the same type. Without them, the type of bond between any two nucleotides could vary. Imagine that an RNA is created in which there is a mixture of bond types between nucleotides. The combination of nucleotide sequence and bond arrangements confers upon this RNA the ability to self-replicate. Unfortunately, there is no way to encode the bond type between nucleotides. In other words, the RNA's offspring will not be identical to the parent, and therefore the offspring will not have the replication ability. Since the ability to replicate is lost after one generation, there is no evolution. Another problem is that an RNA with a backbone made up of different types of bonds is unstable. If an RNA degrades quickly, it is unlikely to replicate fast enough for its copies to spread in the population.
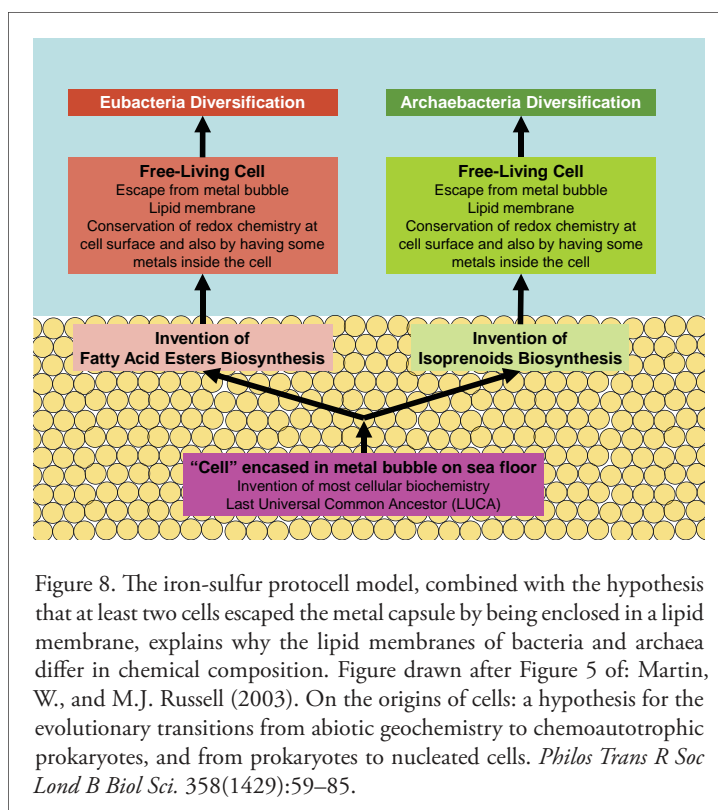

Figure 8. The iron-sulfur protocell model, combined with the hypothesis that at least two cells escaped the metal capsule by being enclosed in a lipid membrane, explains why the lipid membranes of bacteria and archaea differ in chemical composition. Figure drawn after Figure 5 of: Martin, W., and M.J. 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.
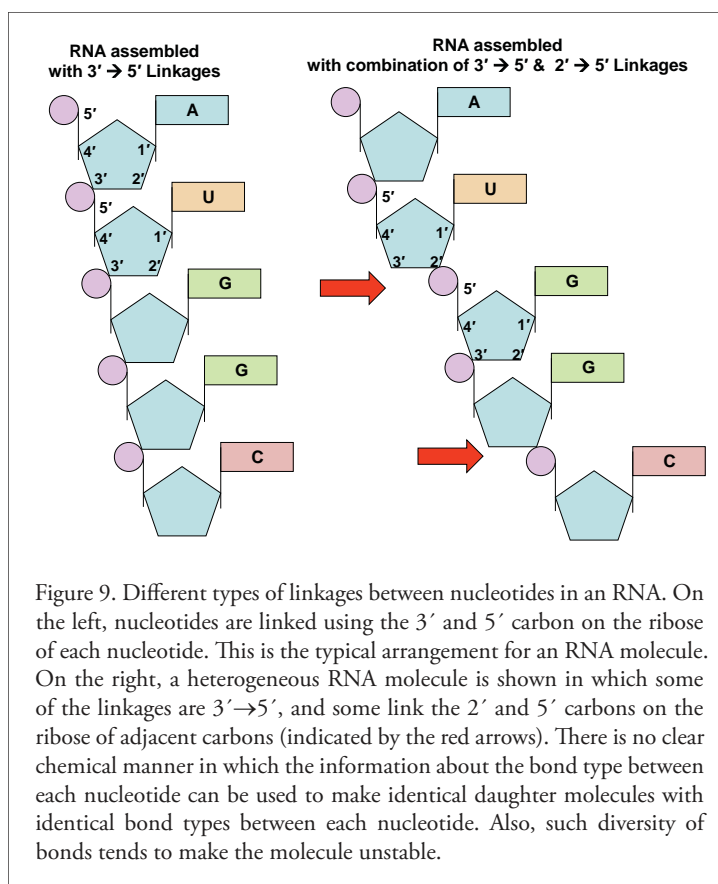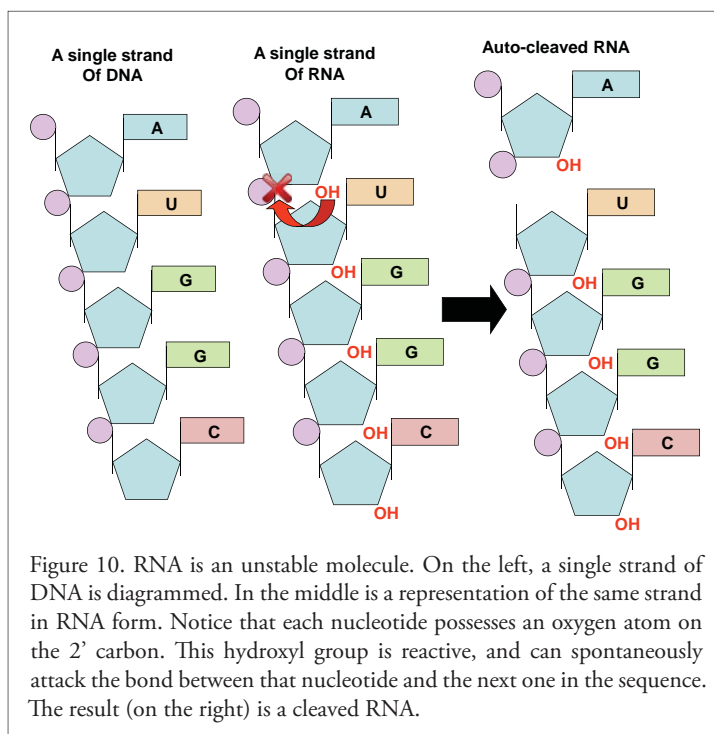

Figure 9. Different types of linkages between nucleotides in an RNA. On the left, nucleotides are linked using the 3′ and 5′ carbon on the ribose of each nucleotide. This is the typical arrangement for an RNA molecule. On the right, a heterogeneous RNA molecule is shown in which some of the linkages are 3′→5′, and some link the 2′ and 5′ carbons on the ribose of adjacent carbons (indicated by the red arrows). There is no clear chemical manner in which the information about the bond type between each nucleotide can be used to make identical daughter molecules with identical bond types between each nucleotide. Also, such diversity of bonds tends to make the molecule unstable.

Third, can RNAs catalyze the necessary replication reactions? Some RNAs called ribozymes have catalytic abilities. Various ribozymes capable of replicating RNA have even been created in the laboratory by artificial selection. However, these ribozymes do not have high processivity (they can only replicate a short number of nucleotides, typically about 20). In addition, they are not very accurate (they often incorporate the wrong nucleotide). 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 faithfully replicate itself. All of these limitations could be due to the fact that we have been studying RNA evolution in the laboratory for only a few decades, while the origins of life may have taken millions of years of trial and error. However, the greatest difficulty may lie in the copying process itself. When an RNA copies itself, it uses the base-pairing rules (the chemical property whereby the base A interacts with U, G interacts with C). The replicated RNA is not identical in sequence, but rather complementary to the template. For example, if the original RNA was 5′ AUG 3′, then the copy is 5′ CAU 3′. Since the sequence of the replicated RNA is different, the copy is not expected to have the same catalytic function as 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.

Also, many protein enzymes in our cells today are associated with a nucleotide in order to carry out their catalytic function. It is argued that these nucleotide cofactors are the remnants of the RNA World. In other words, in the past, RNAs carried out the reactions that these co-factor-needing enzymes carry out today. However, to date, no ribozyme (naturally occurring or evolved in the laboratory) has been identified that can carry out some of the types of chemical reactions that proteins perform easily.

Finally, as RNA is an unstable molecule, could it have persisted long enough to proliferate life? Instability is the Achilles' heel of the RNA World Hypothesis. Even when RNA is properly assembled using all the same linkages, RNA is an unstable molecule. RNA and the more stable DNA differ only at one atom: an oxygen atom on the ribose sugar. This small atom has big consequences for the structure and stability of the molecule. Notably, the oxygens in the RNA attack the bonds that form the scaffold of the RNA polymer, breaking the RNA into its constituent nucleotides (Figure 10). This instability sheds doubt on the ability of RNA to be the first molecule of life.

Figure 10. RNA is an unstable molecule. On the left, a single strand of DNA is diagrammed. In the middle is a representation of the same strand in RNA form. Notice that each nucleotide possesses an oxygen atom on the 2' carbon. This hydroxyl group is reactive, and can spontaneously attack the bond between that nucleotide and the next one in the sequence. The result (on the right) is a cleaved RNA.
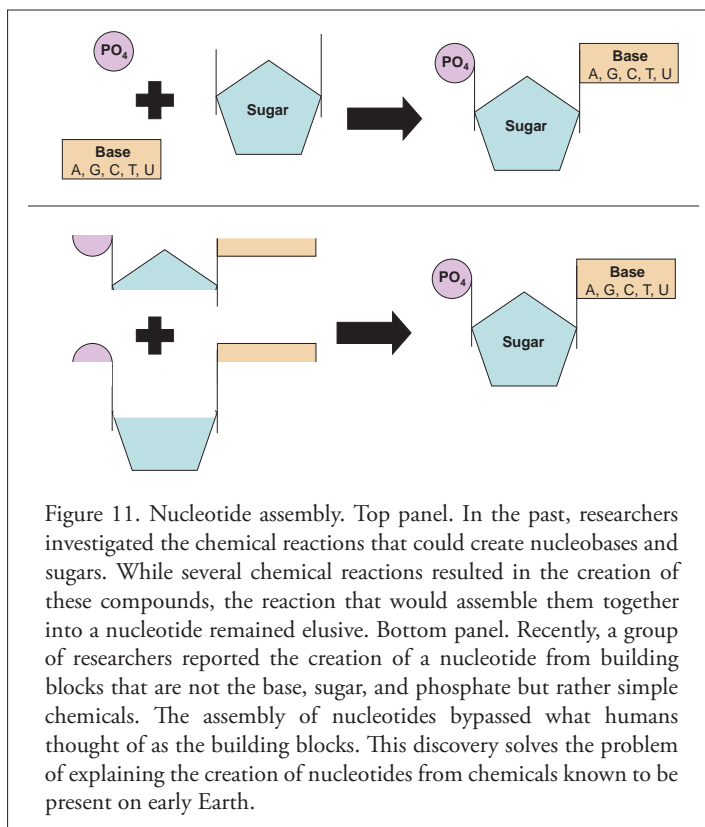
Part III – Team 2: Intimate Debate Handout

Evidence for Replication-First Hypothesis

The possibility of the RNA World Hypothesis lies in the likelihood of several distinct occurrences.

First, could the chemical building blocks that constitute a nucleotide be created by chemical reactions occurring on early Earth? Researchers agree that there are chemical reactions that could create sugars and nucleobases using the inorganic gases and conditions present on the early Earth. Furthermore, it is increasingly evident that these types of chemical reactions can occur in space on asteroids and comets (which rained down frequently on early Earth). Therefore, even if the necessary chemical reactions did not take place here, the basic building blocks could have arrived on our planet from outer space. Thus, the possible creation of sugars and nucleobases from inorganic components on primordial Earth supports the RNA World Hypothesis. However, as these molecules are unstable, they may break down before they can form into nucleotides. Furthermore, for some time the chemical reaction that could bring together a sugar, a base, and a phosphate to form a nucleotide was elusive. This posed a bit of a dilemma, but luckily it was recently resolved. A team of researchers discovered that, using chemicals and conditions found on early Earth, it is possible to build a nucleotide from simple building blocks without first creating the sugar and base (Figure 11). In the past, scientists were looking for a way to first create the building bocks and then assemble them together. However, there is a way, chemically, to skip the creation of the building blocks and go straight to the fully assembled final product. This discovery helps to explain away the instability and dilute concentration problems mentioned above.

Figure 11. Nucleotide assembly. Top panel. In the past, researchers investigated the chemical reactions that could create nucleobases and sugars. While several chemical reactions resulted in the creation of these compounds, the reaction that would assemble them together into a nucleotide remained elusive. Bottom panel. Recently, a group of researchers reported the creation of a nucleotide from building blocks that are not the base, sugar, and phosphate but rather simple chemicals. The assembly of nucleotides bypassed what humans thought of as the building blocks. This discovery solves the problem of explaining the creation of nucleotides from chemicals known to be present on early Earth.

Second, can nucleotides be induced to link together into RNAs? Recent laboratory work indicates that it is possible. The clay montmorillonite, which is a weathering product of volcanic ash believed to be abundant on early Earth, can serve as a catalyst in this chemical reaction. Montmorillonite attracts nucleotides to its surface and holds the nucleotides in the appropriate orientation to favor an elongation reaction and the creation of 30–50 unit RNAs. Another researcher found that frozen sea ice has properties that can also favor the formation of an RNA from nucleotides. Finally, in conditions mirroring those found near hot springs (heat, fluctuation between hydration and dehydration, and in the presence of lipids), nucleotides assemble to form chains up to 100 nucleotides long. There are therefore many possible ways in which RNAs could form from nucleotides.

Third, can RNAs catalyze chemical reactions? In the 1980s, Thomas Cech made the Nobel Prize winning discovery of catalytic RNA, which he called ribozymes (Figure 12, next page). Since that time, the ability of RNAs to serve as catalysts has been confirmed, and the range of chemical reactions that they can facilitate expands every year. Some RNAs can replicate a portion of themselves. However, as nucleic acids replicate by complementary base pairing, the replicated RNA is a negative copy of the original RNA (for example, if the original RNA had the sequence 5′AAG3′, the copy is 5′CUU3′). This poses a problem as the replicated RNA does not fold in the same manner as its parent and therefore does not have the same ability to replicate itself. Its sequence would therefore not be expected to increase in the RNA

pool. So the search goes on for an RNA that has the ability to make an exact copy of itself. This would be the property required for an RNA to be the initial life form. Despite this set back, researchers realize that catalytic RNAs, which are potential cellular relics of an RNA World, carry out many essential cell processes. For example, 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 proteins. 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. Nucleotides can also act as co-factors for many enzymes (e.g., NAD+, FAD, coenzyme A) and ATP, another ribonucleotide, is used by the cell to transfer energy between chemical reactions.

Fourth, does RNA have the ability to attract and link amino acids into a protein? The current existence of tRNA in our cells (which bind specific amino acids to RNA sequences) and ribosomes (which link amino acids together into a protein) demonstrate the possibility. As additional evidence, researchers have evolved an RNA capable of this task in laboratory settings.
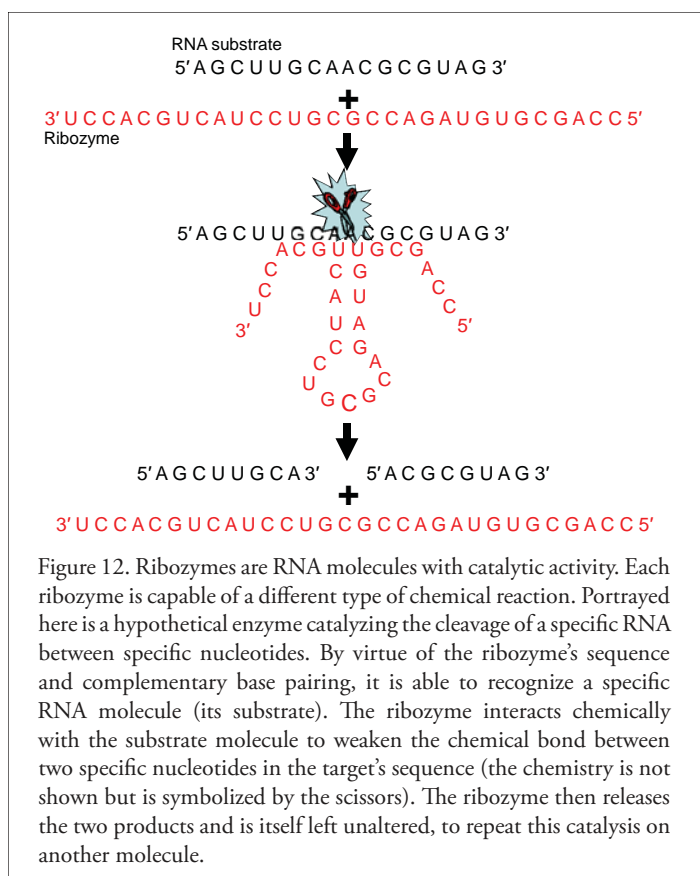

Figure 12. Ribozymes are RNA molecules with catalytic activity. Each ribozyme is capable of a different type of chemical reaction. Portrayed here is a hypothetical enzyme catalyzing the cleavage of a specific RNA between specific nucleotides. By virtue of the ribozyme's sequence and complementary base 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.

Finally, as RNA is an unstable molecule, could it have persisted long enough to proliferate life? Recent evidence suggests that the clay montmorillonite, mentioned earlier in the context of the formation of an RNA, has properties that can shield RNA from decay by UV light. Therefore, clay could have protected RNA as it evolved to create life.

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

The Iron-Sulfur World Hypothesis is a relatively new idea and has not been subjected to as much experimental scrutiny as the RNA World Hypothesis. However, some of the studies that have been carried out cast some doubt on its premise.

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 (expected to be present in the early oceans), these reactions do not take place. This shows that the proposed system lacks the robustness to explain the occurrence of life under conditions of early Earth. Furthermore, simulations investigating the rate at which iron-sulfur catalyzes reactions suggest that this catalyst 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 (when the Earth was constantly hit by space objects and could not support life) and the appearance of cellular life. In addition, at temperatures found near hydrothermal vents, troilite does not easily give its electrons to carbon. Therefore, it is unlikely that iron-sulfur can serve as a catalyst in the biochemical reactions proposed by Wächtershäuser.

Ultimately, the critical question that remains to be answered is this: without any genetic or informational material, how could a metabolic life form reproduce or evolve?