

# Abiogenesis Explained

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## 1 Introduction

We often hear the question how evolution explains the origins of life. The short answer: it doesn't. That is the subject of abiogenesis theory (also referred to as origin(s) of life science). Abiogenesis is basically a hybrid biochemical/geochemical explanation for the origin of life from non-living materials. Evolution is what comes after the first self-replicating system is produced, that is capable of undergoing change. There is, of course, some overlap, when discussing the origins of such self-replicating systems. Here, I'll discuss some basic observations, ideas and experiments that come from abiogenesis studies. Interestingly, some of these methods are also applicable to identifying signs of life on other planets (e.g.: future Mars missions), but I won't go into that in much detail here, except where it is relevant to abiogenesis. I'll start with some basic concepts, after which I will mention some fossil observations of signs of life in the early Earth to give an idea of the top-down approach to studying life through geology and paleontology, and then move on to a bottom up approach which is more akin to biochemistry, in which scientists try to recreate the conditions of the early Earth in the lab, with some successes and some open questions which I'll try to point out. I won't move far beyond the first self replicating systems, as that is where evolution starts, but I'll briefly mention a few key events that lead to the evolution of increasingly complex lifeforms. The details of these, however, are topics that should (and have been) addressed

in other threads. I should note that my knowledge is about 3 years out of date, and that I am merely an interested layman, so new evidence may well have been uncovered recently that I am not aware of. This is a very active and rapidly growing field of research. I have based this summary primarily on a great 2005 introductory video lecture series by The Teaching Company, by Robert M. Hazen (<http://www.teach12.com/>). I have tried to update this information here and there, but there may well be some outdated information remaining.

## 1.1 Spontaneous generation

Louis Pasteur proved that spontaneous generation of life, which before this time had been considered an established fact, was impossible, and that life forming was instead the result of biogenesis (life arising from other life). Before Pasteur's time, for instance, mice were thought to spontaneously appear from stacks of hay. Similarly, micro-organisms growing in colonies on a substance were thought to be born from that substance itself, or from a "life force" in the air. To disprove this idea, Pasteur performed several experiments. He boiled a broth which he placed in vessels that were connected to the outside air through a long, bent tube that would not allow dust particles to pass, as well as some that were completely closed off, and some that were completely open to the air. In addition, he did the same for an unboiled broth. The experimental setup of the first (boiled) experiment is shown in figure 1. Nothing grew in the closed or filtered vessels holding the broth that was sterilized, but something did grow in the vessels holding the broth that was uncooked, independent of whether or not the broth was closed to the outside air or not (he had thereby also discovered anaerobic metabolism). The conclusion was that life did not come from the broth or from the life force in the air, as had previously been suggested, but from other lifeforms carried on spores. Strangely enough, abiogenesis (or origins of life) theory is now trying to establish how primordial life once came from non living materials. Of course, we all know that Earth worms are not born from the Earth, but from other life forms, particularly Earth worms. But at some point, when upholding a scientific naturalist perspective (which cannot comment on the existence or non-existence of God, and thus does not deal with the religious

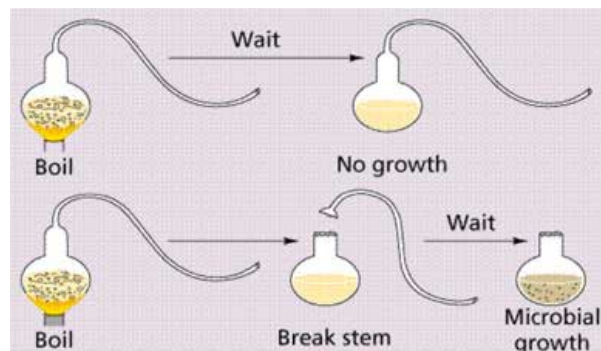


Figure 1: Pasteur's experiment (image from <http://www.angelfire.com/>).

perspective), one has to assume that life ultimately came from non-life. In the end, life is chemistry, and its laws - on the molecular scale - do not differ in any known significant fundamental way from the normal laws of chemistry. Yet life is obviously quite distinct from non living matter. Abiogenesis, then, is not only about the transition from life to non-life, but also about exploring the boundary between the two.

### 1.1.1 Life as a continuum

One of the key questions in this topic is of course the question what life really is. There are many conflicting definitions (almost no two people will have the same definition), but most biologists today now agree on three key properties. This is used as a working definition. First of all, life must be able to grow. Second, it must be able to reproduce. Third, it must be able to undergo reproductive variability (in other words, it must be able to evolve). Under this definition (or perhaps in spite of it), there is thought to be no sharp boundary between life and non-life. Rather, the transition from life to non-life should be seen as a continuum. In that sense, the first self replicating systems are "somewhat alive", but not quite as alive as the first bacterium, or us. I would ask that you keep this in mind while reading the rest of this summary.

### 1.1.2 Modern abiogenesis

Charles Darwin wrote in a letter to Hooker, in 1871, that life began in a "warm little pond, with

*all sorts of ammonia and phosphoric salts, lights, heat, electricity, etc. present, that a protein compound was chemically formed ready to undergo still more complex changes, at the present day such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed.*". According to Darwin, then, it is the present day life which prevents spontaneous generation from occurring today. The modern definition of abiogenesis is quite similar to that suggested by Darwin. Unlike the old definition of abiogenesis, which was, as noted, disproved by Pasteur, and which dealt with the spontaneous generation of complex organisms, the modern theory is concerned with the origins of life from primordial chemicals under conditions thought to have been prevalent on the early Earth. In the rest of this summary, I will discuss only this modern interpretation of abiogenesis. There is currently no broadly encompassing theory, or standard model, if you will, of the origins of life. However, one can find a number of common threads in these models, and all build on discoveries of fossil evidence, and chemical experiments carried out in laboratories. I will start by outlining some of the fossil evidence coming from the "top down" approach to the studies of the origins of life, before moving on to the "bottom up" approach that studies abiogenesis through experimental observations, in attempts to reconstruct early life, using plausible early Earth conditions.

## 2 Fossil evidence of ancient life: constraining time scales

The Earth is thought to be roughly 4.6 billion years old. At first, its surface was red hot, both because the crust had not yet settled, and because it was continuously being battered by fragments of the then forming solar system. These fragments would, on impact, vaporize oceans and throw most of the recently formed atmosphere into outer space, so that no life could survive. The oceans are thought to have formed approximately 200 million years after the formation of the Earth, when the surface temperature was approximately 100C. The Earth remained most likely uninhabitable until the end of the Hadean eon, roughly 4.1-3.8 billion years ago,



Figure 2: The hadean era (image from <http://www.newsback.com/>).

at which time the composition of the atmosphere was very different from that of today. There is some controversy as to the exact contents of this early atmosphere, which we will discuss later on. Here I'll discuss some of the evidence we have concerning time scales. I'm not going to go into fossil dating here - this is not supposed to become a full textbook, but a concise summary. Look into radiometric, and particularly isochron dating if you want more info on this subject.

### 2.1 Microbial fossils

One of the main problems with finding some of the oldest forms of life is that it will have been engulfed, that is, eaten and digested by subsequent generations of organisms. This is of course true for any organism in the chain of evolution, but it is particularly problematic in the case of microbial organisms - and life is thought to start out as extremely tiny, cell-like self-replicating systems, much like a highly oversimplified bacterium. Since most of the really ancient life was both extremely tiny and soft bodied, finding fossil evidence of the (near) first kinds of life is not an easy task. It is rare for soft tissue to fossilize, and even when it does, microbes are hard to spot. The data are sometimes highly ambiguous. Some of the fossil evidence that is *not* so ambiguous, and widely accepted as uncontroversial comes in the form of stromatolites (see figure 3; more info on [wikipedia](https://en.wikipedia.org/wiki/Stromatolite)), which are spherical dome-

like structures that are thought to be composed of mineralized corpses of ancient microbes. These structures have been dated at about 2.5-2.7 billion years old, and there are some other examples of widely accepted fossil evidence that are considered reliable that go as far back as about 3 billion years. There are older known fossils still, but the evidence is often controversial in those cases, arising from such questions as whether or not the fossils in question are actually fossils, and not just natural mineral formations that happen to look like microbes, or whether the rocks were accurately dated. The oldest controversial fossils were dated at around 3-3.8 billion years. Schopf's fossils, for instance, are about 3.465 billion years old, but it is uncertain if what is seen in the minerals is actually microbial in origin. Some images of what he found can be seen in figure 4. Note that uncertainty does not necessarily imply falsehood. If these oldest findings should turn out to be confirmed, this would mean that life formed almost as soon as it was possible for it to do so, which would have major implications for our search for possible extraterrestrial life. A final interesting set of microbial fossils have been found near hydrothermal vents at the bottom of the ocean that are about 3.5 billion years old. I will return to hydrothermal vents later on.

## 2.2 Fossil isotopes

There are other ways of detecting signs of life on the early Earth. Fossil isotopes are specific isotopes that are accumulated into unnatural concentrations by living organisms. For instance, carbon has a number of different isotopes with equivalent chemical properties. The isotopes that differ from the most common one contain one or more extra neutrons in the nucleus of the atom. For instance, carbon, which typically has 6 protons and 6 neutrons, denoted by  $C^{12}$ , can also be found in isotopes with 7 or 8 neutrons, denoted  $C^{13}$  and  $C^{14}$  respectively. We can measure the ratio between different isotopes very accurately using a mass-spectrometer (I'm not going to go into the details of how this works here, but you can look it up if you want to - a simple schematic is given in figure 5). The natural ratio of concentrations between  $C^{12}$  and  $C^{13}$  is about 99:1. Because  $C^{13}$  isotopes are heavier than their  $C^{12}$  counterparts, they tend to be slightly more sluggish in chemical interactions. Because of this,  $C^{12}$



Figure 3: Stromatolites (image from <http://paleontology.edwardtbabinski.us/>).

tends to accumulate in organisms so that the ratio of  $C^{12}$  to  $C^{13}$  is shifted. If there is a 1% excess in  $C^{12}$  from the norm, we say that this constitutes a deviation of *-10 per mil*. For instance, modern photosynthetic life has a per mil of -20 to -30, corresponding to a 2 to 3% excess. Stromatolites are typically in the -25.0 to -25.9 range. Photosynthetic life has a relatively reliable source of energy, so it is typically less efficient at storing carbon than life relying on less stable energy resources. Such non-photosynthetic life can typically be found in the -50 range. In addition, there is a buildup of  $C^{12}$  in organisms higher up in the food chain. So a plant contains a higher excess of  $C^{12}$  than its surroundings, a herbivore contains a larger excess than that plants it eats, and a carnivore that eats the herbivore contains a higher excess than the herbivore. So, in this way, isotopes can tell us something not only about whether some mineral was at some point processed by living organisms (which shows up as the excess deviation from the norm), but it also gives us some basic information about the lifestyle of those organisms (which shows up as the size of the excess). The oldest fossil isotope hint at signs of life comes from an island off the coast of Greenland, and gives dates of around 3.85 billion years.

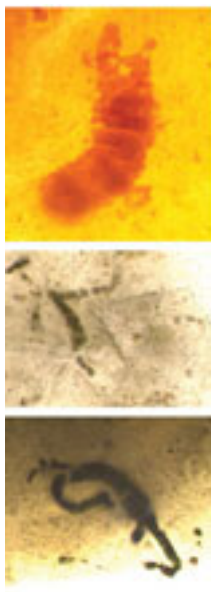


Figure 4: Images of Schopf's fossils (image from <http://www.nature.com/>).

I should note that this evidence was not entirely uncontroversial at the time I heard about it, so it may have been refuted since. But there is other, less controversial evidence that gives estimates in the same basic ballpark figure as the more reliable fossil evidence.

### 2.3 Fossil biomolecules: (a) biosignature molecules

A final type of fossil evidence comes in the form of more complex molecules that are typical to life on Earth today. Life uses only a tiny subset (a fraction of a percent) of the possible carbon based compounds typically found in nature. This is due to the unusual way in which these compounds are synthesized by the cell. So, by looking at the *absence* of compounds *not* found in life in combination with the *presence* of those molecules that are typically found in life, we can identify strong evidence that life was once present at the site of interest. We call these organic compounds biosignature molecules, and we call compounds that are never found in life abiosignature molecules. Both types of signature molecules must be stable over long periods of time

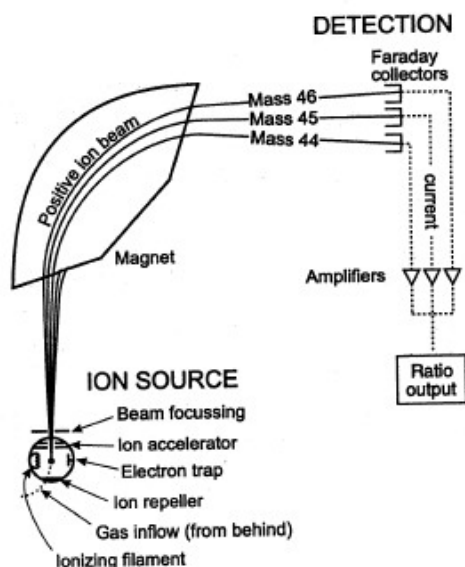


Figure 5: A simple mass spectrometer (image from <http://en.wikipedia.org/>).

and under a large variety of conditions and abundant to the extent that we can easily find traces of them, they must be unique to life or non-life for biosignatures and abiosignatures respectively, and, in the case of biosignatures, they should be essential to life. For instance, hopanes (see figure 6) are carbon molecules that have a molecular backbone composed of several carbon rings of 6 or 5 carbon atoms that are typically produced by the type of synthesis found in cells, and are typically found in cell membranes. Their structure makes them quite rigid and stable, making them a good candidate class of molecules that is frequently considered for this type of investigation. Incidentally, work is currently being done on creating a hopane-detector for an upcoming Mars mission by Steele et al (in the form of MASSE, Microarray Assay for Solar System Exploration). Some of the more convincing identifications of hopanes for which non-biological systems were ruled out as a possible origin are given by Summons, at 2.7 and 2.5 years old. The latter sample contained traces of 2-Methylhopane, which is known to occur only in Cyanobacteria, which are capable of undergoing photosynthesis.

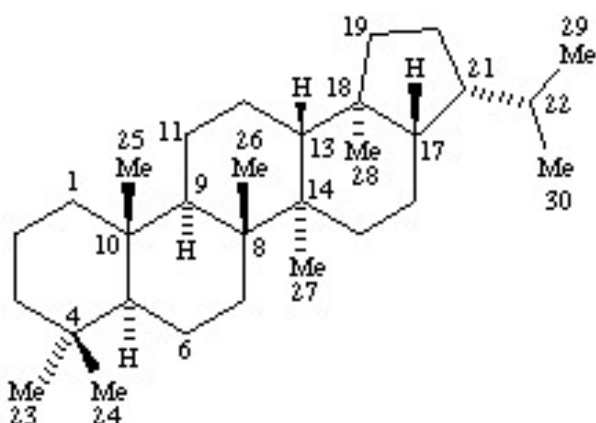


Figure 6: Hopane (image from <http://www.chem.qmul.ac.uk/>).

## 2.4 Primitive aspects of modern cells

One final type of contribution of the top-down approach to abiogenesis comes from molecular phylogeny. I don't want to go into too much detail here, but essentially it concerns the identification of some of the most ancient cellular mechanisms. Basically, what researchers in this field do is look at the correspondences and differences in functionality between the most primitive (note: primitive in an evolutionary context means oldest, not necessarily least complex) and most modern species of organisms. Some mechanisms stand out as rigid and unchanging, suggesting that they play a vital and ancient role for life. For instance, all organisms today seem to require ATP (see figure 7), and so the genes that code for this molecule are more or less fixed in evolution across all organisms. Similarly, as Carl Woese noted, the genes that code for 16S ribosomal RNA (see figure 8) are highly conserved; the correspondence between human beings and e-coli is about 50%. It is expected, then, that one of the most sensible courses of action is to look for circumstances that could have led to the spontaneous development of such rudimentary features of life. The abiogenesis community tends to be divided between two opposing viewpoints. On the one hand, some people promote a "metabolism-first" point-of-view. In this view, the most central part of life is metabolism, the mechanism by which

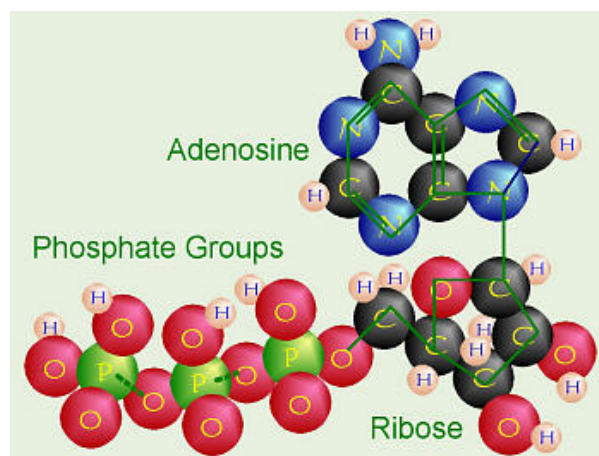


Figure 7: ATP (image from <http://www.dvbiology.org/>).

energy is derived from the environment and put to use for the organism's benefit. Metabolism-first proponents propose that the first self-reproducing system of chemical interactions was a metabolic cycle. Many geochemists working in the field subscribe to this idea. On the opposite side of the debate, the "genetics-first" viewpoint holds that genetic reproduction preceded metabolism. Most biologists tend to support this view. I'll briefly describe the target feature of modern organisms that either viewpoint is focused on at this point in time.

### Primitive metabolism

The metabolism first point of view focuses mostly on one of the most fundamental metabolic pathways that modern organisms employ, named the citric acid cycle. This relatively simple process forms a closed loop of reactions that halves the key molecules at every turn, resulting in the release of energy. Interestingly, the citric acid cycle can also run in reverse, in which case it is called the reverse citric acid cycle, for obvious reasons. In this case, input energy is required, but the reactive loop runs in reverse, duplicating the reactants at every turn. In this way, the cycle can be described as a self-replicating system. I'll describe the reverse citric acid cycle here briefly, because some of the key research focuses on the molecules used in these cycles, particularly pyruvate and oxaloacetate. A simpli-

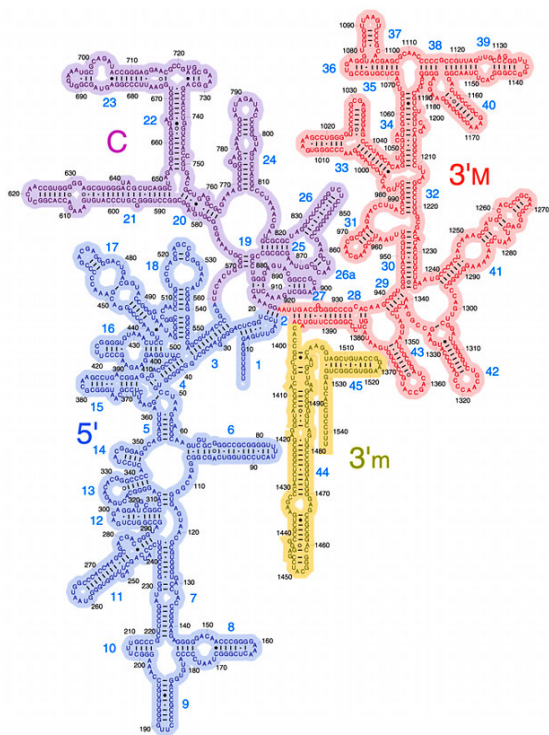


Figure 8: 16S Ribosomal RNA (image from <http://www.learner.org/>).

fied form of the cycle runs as follows, where a + indicates the addition of a certain chemical from the environment and a  $\rightarrow$  indicates a chemical reaction resulting in the molecule to the right of the arrow, starting from Acetyl-CoA: Acetyl-CoA, +  $CO_2 \rightarrow$  Pyruvate, +  $CO_2 \rightarrow$  oxaloacetate, +  $H_2 \rightarrow$  Malate, +  $H_2O \rightarrow$  Fumarate, +  $H_2 \rightarrow$  Succinate, + CoA  $\rightarrow$  Succinyl-CoA, +  $CO_2 \rightarrow$  2-Oxoglutarate, +  $H_2$  &  $CO_2 \rightarrow$  Isocitrate, +  $H_2O \rightarrow$  Aconitate, +  $H_2O \rightarrow$  Citric Acid, + CoA  $\rightarrow$  Acetyl-CoA & Pyruvate. The (normal) citric acid cycle is shown in figure 9. As one can see, the last step that splits a citric acid molecule doubles the amount of oxaloacetate, which leads to a doubling of the entire cycle, and so this metabolic pathway can, in principle, be self-replicating. One major problem is that, in modern cells, this process requires many complex enzymes (catalytic proteins) which were almost certainly not around in a pre-biotic era. We will see later how this paradoxical situation may be resolved by the

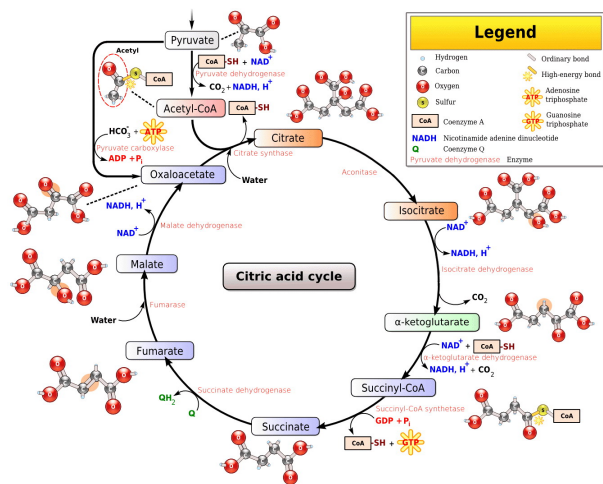


Figure 9: The citric acid cycle (image from <http://www.biologycorner.com>).

use of minerals as catalysts.

### Primitive genetics

As we all know, genetics deals with the macromolecules (large molecules, also referred to as polymers, which are generally built up of smaller basic molecular units, called monomers) in an organism's cells that hold (and in some cases process) the information for its development and functioning, namely DNA and RNA, and possibly proteins. It may serve us well to run through the basics of proteins, DNA and RNA here, so I'll do that briefly. Both DNA and RNA are made up of long chains of four alternating different smaller molecules called nucleotides, where each nucleotide contains a distinct base (generally a carbon ring structure), a phosphate group, and a backbone (which links the nucleotides together in a strand). The bases each line up with one (and only one) of the other bases (as seen in figure 11), so that two mirror images of two different pairs can be formed (so four base pairs in total). Where DNA uses the bases thymine (T), adenine (A), glycine (G) and cytosine (C) (where C can bind to G, and A to T), RNA uses uracyl (U) instead of thymine (so that A binds to U). The structures of the five bases and a comparison between the structures of DNA and RNA are shown in figure 10. Both RNA and DNA replicate by use of complementary base-pairing, where each nu-

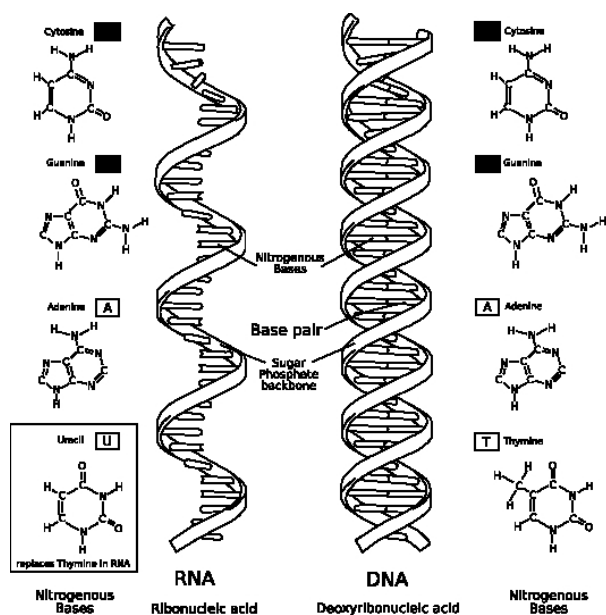


Figure 10: Comparison between DNA and RNA (image from <http://en.wikipedia.org/>).

cleotide is paired up in sequence with its complementary base (see below), producing a complementary sequence. In RNA, the new sequence then splits off from the old one to produce a copy. Note that a copy of RNA is a complementary strand, much like a cast, which can serve as a template for the final, actual copy. The only way in which RNA can replicate in one step is if the strand is palindromic, i.e. has the same sequence of bases when read in either direction. When a sequence of nucleotides is its own complement in this way, we call it self-complementary. DNA has to unwind and be split down the middle for the copy process to take place (this is achieved by various proteins), after which both strands are completed by adding their complementary strand to the now naked DNA half. There are a few more differences between DNA and RNA. First of all, while DNA consists of two strands (figure 10, right), RNA consists of only a single strand (figure 10, left). Second where DNA uses a backbone made up of a sugar called deoxyribose (the D in DNA), RNA uses a slightly different sugar named ribose. Finally, the main thing to note about proteins is that they are made up of sequences of molecules called amino acids. The

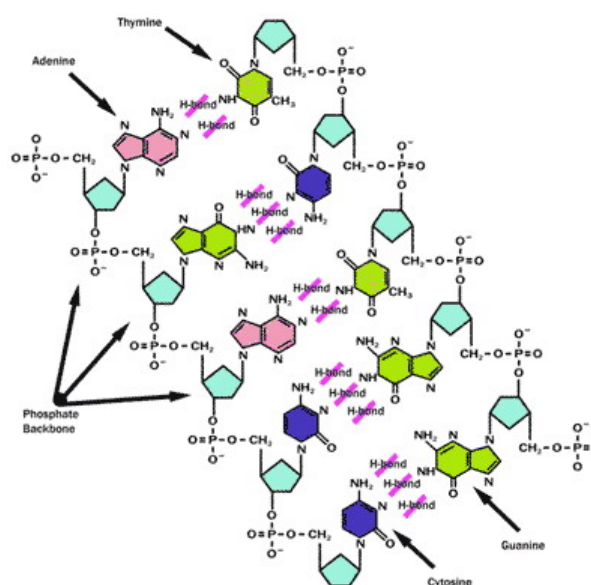


Figure 11: Base pairing and chemical structure of DNA (image from <http://www.molecularstation.com/>).

sequence of amino acids is coded for by DNA or RNA, and this determines the protein's form and (thereby) function. Proteins are the work-horses of modern day cells. From a genetics-first abiogenesis point of view, the first self-replicating system consisted of one (or, less likely, a network) of these molecules. Broadly speaking, there are 4 possibilities, as outlined by Orgel:

1. A self replicating peptide: This is somewhat appealing, because amino acids, which are the basic building blocks of peptides, were (as we will see) likely to be readily available in the pre-biotic environment. By contrast, the synthesis of nucleotides (the building blocks of DNA and RNA) has proven to be more problematic. We will see that Fox proposed a model of this type. There are, however, two basic problems with this view. First of all, peptides would somehow need to “invent” DNA or RNA. Second, the linking of amino acids into peptides is a messy process without the aid of the many proteins and other regulatory systems present in modern cells.
2. The simultaneous appearance of DNA and pro-



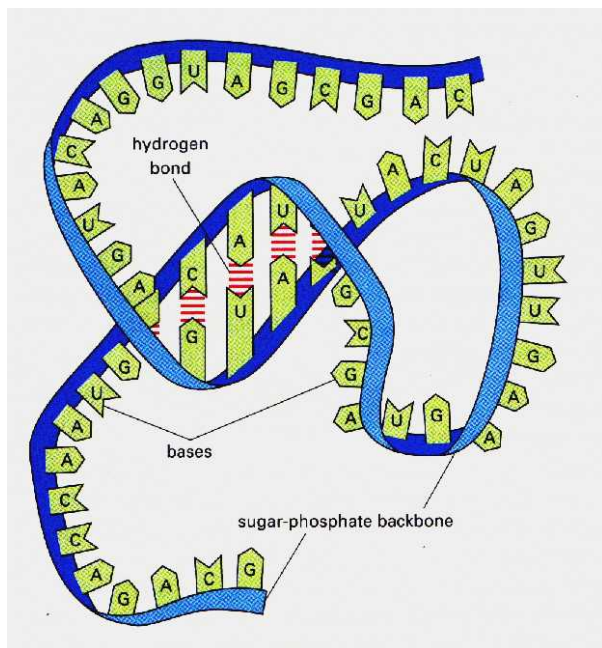


Figure 12: Folded RNA strand (image from <http://www.uic.edu/>).

teins: Because proteins are required to unfold and copy current-day DNA, we may have to assume that they were likewise linked in the past. For this reason, both DNA and proteins would have to appear at the same time. This possibility is obviously unappealing, because it requires the simultaneous occurrence of two complicated steps of macromolecule formation. It presents us with a paradox; in modern cells, DNA is used as the building plan to make proteins, but proteins are crucial in copying DNA.

3. A clay world model, which we will describe in some detail later on.
4. A model in which a nucleic acid similar to RNA acts as both an information carrier and a catalyst that promotes self-replication: This model could, according to Fox, be studied most easily in the laboratory. It is also the most likely alternative for various other reasons. First of all, RNA, in the trinity form of mRNA, tRNA and rRNA, is one of the most essential core molecules of ancient genetics, in much the same way that the citric

acid cycle is central to metabolism. Before the 1980's, people had thought that all enzymes in a cell were catalytic proteins. But in the early 80's, both Altman and Cech independently found an enzyme that consisted entirely of RNA. This means that RNA can both contain information and catalyze important reactions (like self-replication). This thus resolves the DNA/protein paradox.

RNA plays many important roles in all modern cells. For instance, mRNA is the result of transcribing a DNA strand, and tRNA and rRNA are subsequently responsible for the translation of mRNA into amino-acid chains that we call proteins. Additionally, RNA nucleotides play structural roles in proteins called co-enzymes. These co-enzymes promote reactions in, among other things, the citric acid cycle, including the production of citrate from oxaloacetate, as well as in helping to build lipids (which are the building blocks of cell membranes) from other essential biomolecules. Finally, RNA can act as chemical sensors in the form of so-called riboswitches, which change shape when they come into contact with other chemicals. This makes the central and ancient role of RNA very plausible. RNA is therefore considered the prime candidate information bearing molecule. As we shall see, the construction of its nucleotide parts has been problematic. However, there are interesting hypotheses as to how this problem may be resolved. Particularly, there may once have been close relatives of RNA that have now gone extinct, which rely on the same nucleotide bases as RNA, but which use a different backbone. A top-down approach to studying RNA that is worth mentioning involves taking existing prokaryotic cells, and engineering them with progressively fewer genes, so as to identify the minimal genetic requirements for life. John Desmond Bernal called this process biopoesis.

### Chirality

A final curious feature of biomolecules is their so-called chirality, or handedness. Most (if not all) biomolecules come in mirror image pairs, that are referred to as right-handed (abbreviated by D, for dextrose=right) and left-handed (L, for livo=left) varieties. These pairs are called chirals (the different instances are called enantiomers). They form because of the tendency for carbon molecules to

form 4 bonds. An example of chirality can be seen in figure 13. Most of the properties of chirals are exactly the same. For instance, they have the exact same composition and structure (except in a mirror image of one another) and many of the same physical and chemical properties. However, life tends to use only one chirality of each general group of molecules (this is called homochirality). For instance, it tends to use only right handed sugars, and left handed amino acids. Since both chiralities are equally distributed throughout nature, it is something of a mystery why only a single chirality is used. However, it has become clear that this reliance can not be violated without cost; cells respond differently to different chiralities. Proteins can become non-functional (because of the fact that protein function is for a large part determined by shape) and the DNA helix (the familiar spiral shape) a mess (because the backbone becomes deformed) without chiral purity. This can result in anything ranging from different responses of taste receptors to birth deformities. All of this suggests that pre-biotic synthesis was inherently asymmetric, resulting in homochirality. We will later discuss some hypotheses about why this may have happened. An interesting side note about chirality is that it tends to flip every few thousands of years. By knowing the ratio between different chirals in current day life, fossils can therefore be dated by measuring the increase in the non preferential chirality. For instance, when an organism dies, it will have only L-amino acids. Over time, increasingly more of these flip into D-chirals until a 50/50 equilibrium is again reached, and an age estimate can be given on the basis of the ratio of L to D amino acids. However, chirality can also be influenced by environmental factors, such as acidity.

I will close this part with a few more notes from phylogenetic research. One of the key things to note here is that microbes called prokaryotes (bacteria and archaea are prokaryotes), which are much simpler than eukaryotes such as ourselves, are thought to be much older than the eukaryotes. The archaea, which are a distinct from the bacteria, seem to be autotrophic, rather than heterotrophic. That is, they are capable of making their own building blocks (their own food) and deriving their energy from (an)organic chemical sources, rather than from sunlight (for example, plants are heterotrophic, by way of photosynthesis). It is pos-

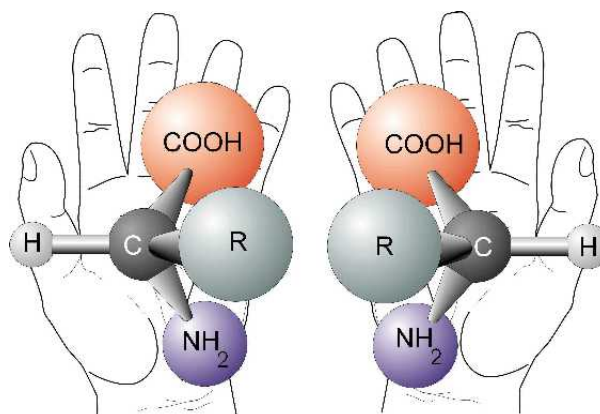


Figure 13: Chiral molecules (image from <http://www.ehu.es/>).

sible that these autotrophes evolved from surface heterotrophes, but that, as crust-dwelling microbes (which are often referred to as extremophiles, and which we will meet again later on) were better protected from comet impacts or other natural disasters, their ancestral heterotrophes became extinct and the autotrophes became dominant by default. A final thing to note is that phylogeny of prokaryotes is often complicated by their tendency for horizontal (or: lateral) gene transfer, in which two individuals sometimes swap DNA. Because of this cross linking, it is practically impossible to determine traits of the so-called last common ancestor (the lifeform from which all current life is thought to be descended) purely by phylogeny.

### 3 Bottom Up: Emergence and abiogenesis experiments

The top-down approach to identifying or constraining the origins of life is useful for giving some time scale constraints, but it is inherently limited because of the inevitably limited amount of information we can deduce from it. Another, bottom-up approach is to try and simulate plausible conditions of the early Earth or solar system, and try to synthesize primitive biochemical compounds. This field is known as pre-biotic chemistry. Of course, we cannot say with absolute certainty that what we find is exactly how things went down on the early Earth, but as long as we use a plausible range of conditions, these experiments, if successful, make the case that life can indeed form spontaneously from non-life, and that it does so under circumstances that are known to occur out in the natural world. I'll start with a very brief description of the basic notion of emergence, on which most of this work is based in the end, and will then move on to increasingly complex experimental results, and the underlying hypotheses of the experiments.

#### 3.1 Emergence

Emergence (a term coined by G. H. Lewes) is a field of study that basically addresses the question of how nontrivial patterns arise from the interaction between simple agents. One can think of the interaction between sand grains and water that creates different characteristic patterns of bumps and ripples on a beach, the interaction between neurons from which consciousness emerges, or a complex chemical pathway that gives rise to emergent patterns in the resulting environment. As far as I can tell, emergence and chaos theory (particularly the field of synchrony, pioneered by Art Winfree) seem to be intimately connected, and may well be two sides of the same coin. Scientists have identified a number of key factors in an emergent system. The density of the agents, that is, how concentrated they are in a given environment, is the first. There are critical densities that, when crossed, give radically different behavior from lower or higher densities. In abiogenesis, this translates into the notion that there is some minimal concentration of the bioparticles present for the formation of more com-

plex molecules and molecular systems. Secondly, the ways in which these agents are interconnected are of great importance, both in terms of the types of interaction, and the degree of connectivity. An example is the way in which neurons are connected in the brain, or, in case of abiogenesis, the ways in which the different chemicals in the environment can interact with one another. A third factor is the flow of energy through the system. There is some critical amount of energy flow for which a system gives rise to emergent behavior. Too low a flow, and nothing happens. Too high, and the emergent behavior is quickly reduced to rubble by the energy influx. Finally, the way this energy cycles through the system is important in both the type of flow, and the rapidity of it. I will return to these points at the end of the next paragraph. Overall, these properties of or precursors to emergence will be evident in the subsequent paragraphs.

#### 3.2 The spontaneous generation of simple organic molecules

We now know that simple organic molecules, duped monomers, such as amino acids (which are the building blocks of proteins), lipids (the building blocks of cell membranes), sugars and bases (the most integral parts of the building blocks of the nucleotides that make up DNA and RNA) can form spontaneously under a variety of circumstances. Cocktails of such molecules tend to be referred to as the pre-biotic or primordial soup, a term that was coined by Oparin. I'll discuss the most prominent of these hypotheses here. The story is very complex and involves a number of models, all of which are capable of producing monomers, and some even produce polymers, which are generally made up of strands of many monomers (but we'll get to that in the next section). But this first part of the story turns out to have one very simple answer: it is easy to spontaneously synthesize most of the basic building blocks of life under a huge diversity of circumstances.

##### 3.2.1 The Urey-Miller experiment and primordial soup

The most famous experiment that demonstrated the spontaneous generation of monomers was the Urey-Miller experiment, which I'll describe here.

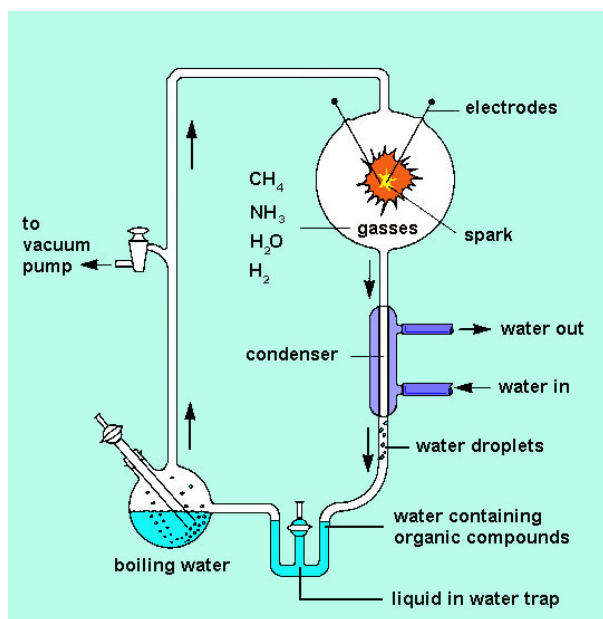


Figure 14: Urey-Miller experiment setup (image from <http://www.uic.edu/>).

Since this experiments, some concerns have been raised with regard to the validity of the underlying assumptions regarding the early atmosphere, but subsequent experiments have shown that monomers also arise under a large variety of different circumstances. I'll close this paragraph with the main questions that challenge the Urey-Miller results. Miller, for his PhD thesis, simulated the early atmosphere of the Earth as conceived by Urey, his adviser. Urey postulated that the early Earth would have an atmosphere that is radically different from today's. Today's atmosphere consists mostly of Oxygen ( $O_2$ ) and Nitrogen ( $N_2$ ), but the high dose of oxygen is mainly due to the process of photosynthesis carried out by plants. Urey hypothesized that the early atmosphere would have been highly *reducing*, which basically means that it prevented oxidation by the removal of free oxygen from the air. In particular, Urey hypothesized that the early atmosphere was composed mainly of hydrogen ( $H_2$ ), Methane ( $CH_4$ ) and ammonia ( $NH_3$ ). Miller set up a simple and elegant experiment in which he heated up water, resembling the Earth's oceans, passing the water vapor through a series of tubes into a vat containing the atmo-

spheric gasses (simulating the atmosphere itself) as described by Urey, and adding series of electrical sparks as an energy source to generate a chemical reaction (basically simulating lightning). In essence, the energy blasts electrons away from the chemical compounds, making them more reactive. It should be noted that subsequent experiments have also explored other energy sources, such as UV radiation, as well as other atmospheric conditions, with similar results. The water vapor, mixed with the chemical compounds resulting from the gas, was then condensed through a series of tubes leading back in to the "ocean". The basic setup of the experiment is shown in figure 14. After only a few days, Miller found that his mixture had synthesized about half a dozen amino acids, among other things. The experiment was confirmed independently a number of times, and was also repeated with variations thrown into the mix, using, for instance, a different mixture of atmospheric gasses, a different energy source, or an addition of powdered minerals (representing soil). Almost every kind of monomer used in current-day life has been synthesized in this way, with three notable exceptions: ribose (a sugar), and the nucleotide bases adenine and guanine. It is interesting to note that the Urey-Miller experiment gives a similar distribution of monomers to that employed by life today - though it should be noted that the experiment also produced many other molecules that have no role in current-day life. What basically happens in the Urey-Miller type experiments is that, under the influence of energy, the atmospheric gasses form a highly reactive mixture of chemicals like hydrogen cyanide ( $HCN$ ) and formaldehyde ( $CH_2O$ ), which easily bind to other molecules in the environment. For instance, amino acids are made when  $HCN$ ,  $CH_2O$  and  $NH_3$  undergo what is known as Strecker synthesis. John Or found that when a solution of  $HCN$  was heated, adenine was produced. Similarly, in a rich solution of  $CH_2O$ , sugars, including ribose, were spontaneously produced. The problem was that Miller's concentrations of  $HCN$  and  $CH_2O$  were typically too low to produce the reactions. Essentially, adenine is produced when 5  $HCN$  molecules combine, and in Miller's solution the largest chains of such molecules were length four  $HCN$  chains. It turns out, though, that there is a solution to this problem. Orgel proposed that, when water freezes that contains a solution of

molecules, the thing that freezes most rapidly is the pure water. The solution therefore tends to become more concentrated during the freezing process, and this can then give rise to interesting chemical interactions. This is an example of emergence where the energy flow has an essential role to play. These reactions are slowed down, however, because of the decrease in temperature. When Miller heard of this, he decided to freeze his solution to -20C to see what happened. Apparently he forgot about it, because it remained in his freezer for about 20 years, making it one of the longest lasting experiments in the history of chemistry. After finally retrieving the sample, it was found that large amounts of adenine had indeed been generated. The importance of this is that it suggests that, although the ancient sea may in itself have been too diluted to account for all monomer occurrences, as the Earth went through subsequent periods of freezing and heating, the monomers that function as the basis of organic chemistry could have formed quite easily.

There are four basic problems with the Urey Miller experiment. First of all, there are some serious doubts about the composition of the simulated atmosphere used by Urey. More recent studies from geochemistry suggest that the atmosphere was a less reactive atmosphere of mostly N<sub>2</sub> and CO<sub>2</sub>, lacking in CH<sub>4</sub> and NH<sub>3</sub>. Secondly, although monomers were formed, almost none of the polymers (like RNA and proteins) were found, and these polymers really form the basis of functionality in modern living cells. Third, according to Brooks and Shaw (1973), there is no evidence in the geological record that any primordial soup existed; that is, if it had, we should expect to find sedimentation that confirms this, but we have never seen anything of the sort. Finally, these polymer macromolecules tend to break down under Miller's conditions, both because of hydrolysis and when subjected to high doses of energy, like the electricity or ultraviolet radiation used in these experiments. Hydrolysis means that, when immersed in water at room temperature and pressure, peptide bonds, which hold together chains of monomers in many kinds of polymers, tend to break down, as they are not as strong as covalent bonds (bonds that are formed by sharing of one or more electrons). When peptide bonds are forged in the process known as condensation polymerization, they release water, and inversely, when in the presence of lots of water, these bonds

are easily broken down. This has to do with the polarized nature of water; an H-bridge, which is responsible for such bonds, is formed because water molecules are not radially symmetric. The result is that one side of the molecule has a slight positive charge, while the other side has a slight negative charge. The molecules can line up head-to-tail and form weak bonds between the positive and negative ends, which is, in a nutshell, what also leads to water surface tension. Also, the Miller experiment produces many chemical components that would cross-react with the amino acids or break up any forming peptide chains. Hydrolyzation is a problem that needs to be solved, which plays a major role in many abiogenesis hypotheses, and it again shows to importance of energy for emergent behavior. Subsequent experiments have gone some way in addressing these concerns, and I will discuss them further below.

### 3.2.2 Extremophiles

It was once assumed that most life on Earth concentrates at or near the surface of the crust that coats our planet, under circumstances that we find the most familiar. However, this turns out to be an erroneous assumption. For one thing, the discovery of life in the deep ocean near submerged volcanic systems known as hydrothermal vents suggests that life can exist under much more versatile conditions than previously assumed. Secondly, following the discovery of life near these vents, discoveries have been made in recent decades (most of which since the early '90's) that suggest that nearly half the Earth's biomass (the combined mass of all living organisms on the planet) may be found in subterranean microbes (particularly archaea, which are thought to have been around at least as long as, and possibly longer than bacteria) that live deep within the crust, at depths of up to at least five kilometers. They can be found inside deposits of granite, basalt, and other minerals that we generally consider very inhospitable. Furthermore, living microbes have been found under the intense heat of volcanic areas, or under a mile of Antarctic ice. Basically they are found almost anywhere wherever there is water present. These organisms live under circumstances of extreme pressures and high temperatures, lacking in any significant amount of sunlight, circumstances under which most surface



Figure 15: A hydrothermal vent. Note the tube worms at the left of the image (image from <http://web.uvic.ca/>).

life would be unable to survive, and they are therefore generally called “extremophiles”. They tend to have an extremely slow metabolism and may be inactive for thousands of years. Cell division may only occur once every millennium, and for the remainder of the time, these organisms seem to just sit and wait. This is particularly true for the crust-dwelling and ice-dwelling microbes (but less so for those near vent-systems). There appears to be little or no predation in these systems, and so the main source of competition is competition for resources. Extremophiles have been an important factor in the proposal of several different alternatives to the Urey-Miller source of monomers. Although they are generally referred to as competing hypotheses, we can view these as many simultaneously (or successively) productive sources of basic organic matter. I will discuss some of the more prominent of these in the next few paragraphs. One of the fundamental characteristics of these environments is that their extreme conditions can give rise to unexpected chemical reactions, yet another example of emergence.

### 3.2.3 Monomers from hydrothermal vents

The idea that the sun is the prime source of energy that ultimately feeds life on Earth is true for most of the life that currently exists on Earth. Miller’s experiments are therefore based on the idea that life originated at or near the surface of the

oceans. However, as we’ve seen there are some potential problems with a theory of this kind. Another hypothesis came along with the discovery of (by today many) isolated complex self-sufficient ecosystems around hydrothermal vents at the bottom of the Atlantic and Pacific oceans (sometimes referred to as “black smokers” when they emit clouds of black material), which are almost completely cut off from the sun as a prime source of energy and exist under crushing deep sea pressures (500-2000 atmospheres) at hot temperatures (200-300C). Hydrothermal vents are deep-ocean cracks in the Earth’s surface from which mixtures of heated gasses are emitted. Vent structures consist of microcaverns that are coated by thin, membrane-like metal sulfide walls. An image can be seen in figure 15. The current-day ecosystems surrounding these vents contain microbial organisms, as well as species of crabs, shrimp and tube worms. It turns out that the microbes are the primary energy producers in these systems, reminiscent of the role of plants in more familiar ecology. These organisms exploit the mineral instability that results from the hot water and gasses emanating from the deep sea vents, mixing with the cold water surrounding them, and flowing over sulfur-rich surface minerals in the process. It has since been proposed, by Hoffman, Baross and Corliss, that hydrothermal vents may well be the best place for the formation of early life. The deep ocean would have provided more shelter from comet impacts that the early Earth was subjected to during the Hadean eon, and the fossil evidence mentioned earlier, and the observation that current day ecosystems thrive near these vents provide strong support for this hypothesis.

There appeared to be some potential problems here that needed to be addressed that Miller, as a defender of an opposing view, was obviously keen to point out. First of all, as was the case in the Urey-Miller experiment, the heat from the vents was thought to break down any macromolecules that are formed. But, as we will see in a later paragraph, this assumption turns out to be likely to be false (or rather, incomplete and therefore inaccurate). Second, modern day hydrothermal vent ecologies are dependent on oxygen, which ultimately comes from photosynthetic (plant) life. The early Earth would have been lacking in free oxygen. In other words, there would have to be a way for a rudimentary metabolism to evolve that did not require

large amounts of oxygen. So, the hydrothermal vents theories have their problems, but they opened the door for alternative hypotheses from the thus far unrivaled primordial soup idea. We will return to these issues when we look at the emergence of polymers and self-replicating systems. Hydrothermal vent discoveries also kick started the search for other extremophile organisms, as we mentioned above.

### **Monomers from minerals**

Up until now, we have focused mostly on the role of the ocean water and the atmosphere, and have largely ignored the role of minerals in the Earth's soil. Obviously, minerals and rocks may have played an important role. First of all, as a source of protection, overhanging rocks can shield forming organic molecules in a tidal pool from the UV radiation of the sun. Second, they can protect tidal pools from incoming waves, and, as water evaporates, allow such a pool to become condensed, resulting in a less dilute soup of organic components, which is more likely to undergo reactions and polymerize. Similarly, organic molecules can accumulate in small pockets in, for instance, volcanic rock, which keep them condensed as water evaporates from them, as well as protected from UV light. Additionally, many of the most common rock faces contain multiple cracks and pores roughly the size of a cell. Such pores and cracks result in an enormous surface area on which many simultaneous natural "experiments" for self-organization can occur. Finally, it makes sense that water, Earth, and atmosphere, as the Earth's key ingredients, played a cooperative role in the formation of life. This section will outline several theories of monomer (and some possible polymer) production that revolve around minerals, rocks, clays and crystals. As we will see, minerals have a number of important properties that allow them to function as catalysts, sources of energy, sources of protection, and as scaffolding for the construction of larger molecules that are not stable enough to form spontaneously.

### **Hydrothermal vents revisited**

Hydrothermal vents remain one of the prime candidates for the place of the origins of life, and we will encounter it several more times, most notably in Günter Wächtershäuser's metabolism-based iron-

sulfur world, and in the PAH hypothesis for the origins of RNA. Therefore, I'll mention a number of preliminary experiments on origins of monomers here. Many (though not all) of these experiments incorporate minerals as key ingredients. First, Hazen, Morowitz, Yoder and Cody performed several experiments under high pressure, incorporating a realistic mixture of powdered minerals, atmospheric gasses, and water. The initial motivation for this came from the observation that the dielectric constant of water, which is a measure of polarity that influences the ease with which peptide bonds can form, decreases dramatically under high pressures and at high temperatures, from about 80 to 20. Recall that, at normal pressures and temperatures, water acts as a(n unusually potent) natural organic solvent which can easily break peptide bonds, and that this has to do with the polarized nature of water. By decreasing the dielectric constant, then, it may be possible for peptide bonds to form. Hazen et al. decided to concentrate on the reverse citric acid cycle described earlier, particularly on pyruvate. Recall that pyruvate plays a major role in this metabolic cycle. It also plays a fundamental role in multiple other processes, such as the splitting of glucose into 2-pyruvate (a process known as glycolysis). Pyruvate is essential for life as we know it, but it does not work in water at room temperature without a catalyst such as an enzyme, and it tends to break down. To test their hypothesis, they subjected a mixture of pyruvate and water to intense pressure and high temperatures similar to those found at hydrothermal vents. The result was that pyruvate did indeed react, in a big way. In fact, their experiment resulted in so many different chemicals (tens of thousands) that it was impossible to analyze in full; a hopelessly diverse mixture referred to as "humpane". What they found was that many alcohols, sugars, and various larger molecules that resemble those found in biochemistry were synthesized (showing both ring and branching structures), and that polymerization had occurred in a variety of molecules, some of which incorporated dozens of carbon atoms. However, this abundance also posed the question of where to go next; with such a large variety of ways, it is almost impossible to predict which roads are the most promising. There are a few potential problems with these experiments, which can be summarized as follows. First of all,

the concentration of pyruvate used was unrealistically high, and the end products too diluted for continuous chemical interactions. So, for this theory to work, a way must be found by which both the reactants and the end products can accumulate (and stay accumulated) closely together. Second, similar to Miller's predicament, the reactions resulted in a large number of products, many of which play little or no role in life. The problem is then how we can explain how particular products may have been selected, and others excluded in the formation of life. Third, many organic molecules were still missing, and so other ways would have to be found to account for these.

But several more experiments have since been conducted. One of these experiments, by Jay Brandes, featured a mixture of water, nitrogen, and iron rich minerals (commonly found near vents), resulting in ammonia ( $NH_3$ ; recall that Miller assumed this to be present in the atmosphere; an assumption that later turned out to be false). Ammonia is an essential ingredient for amino acids. This result suggests that hydrothermal vents may be principle sources of ammonia. A follow up experiment combined ammonia, pyruvate, and several common powdered minerals, resulting in (among other things) large quantities of the amino acid alanine. This directly contradicts Miller's criticism that vent-type conditions would destroy compounds like amino acids. Additionally, Brandes revealed in a later study (on the amino acid lucene) that at least some (and possibly all) amino acids are much more stable in the presence of the mineral pyritite (iron sulfur), which is often found near vents. As we will see, the minerals used in these experiments are likely to be very important in understanding the origins of life. Similarly, studies on bone fossils have revealed that certain minerals can prevent (to some extent) the rapid breakdown of protein structures, because of strong bonding between the minerals and the proteins. This protects and preserves them, and this can work for amino acids as well. The possible "soft tissue" recently discovered in fossil T-Rex bones by Mary H. Schweitzer may have been preserved in this way. In a later experiment, Kono Lemke and David Ross showed that, when glycine and water were cooked under vent-like conditions (without the addition of minerals), glycine declined much more slowly than under normal conditions. What was

even more surprising is their discovery that this also lead to the rapid link chains of amino acid formation. This contradicts the common knowledge that these chains are destroyed by high temperatures (another example of emergence). Under these conditions, peptide chains are much less soluble and therefore more stable. If these form rapidly near vents and then float out in clumps, into the cooler sea water, they separate out as a much more stable second-phase product. This is quite significant, because it gives a partial possible solution to the macro-molecule construction problem. Carbon fixation reactions, in which more carbon atoms are incorporated into an organic molecule to form larger molecules, is common and happens rapidly in hydrothermal experiments. Two of the most common pathways can be described as follows. The first is promoted by many common minerals that incorporate iron, zinc and/or copper. These minerals promote the so-called Fisher-Troph synthesis, which is a carbon fixation reaction which results in chain-like molecules. These results have been confirmed by studies at real current-day hydrothermal vents, and the resulting products are similar to those found in petroleum. The second pathway is driven by cobalt and nickel sulfurs, and promotes a so called CO-insertion reaction, a carbon fixation reaction in which carbon monoxide is inserted. If one repeats these reactions and mixes the results, many complicated molecules can easily be synthesized reliably. Finally, minerals often dissolve at high temperatures and pressures, resulting in chemical reactants that can act as both catalysts and reactants. For instance, sulfur, can dissolve and react with water and  $CO_2$  to give rise to thiols and thioesters, which are catalysts for additional biochemical pathways (we will discuss these later in the thioester world hypothesis). Similarly, iron, water and  $CO_2$  can form so-called iron complexes, structures which can act both as catalysts and reactants (see the iron-sulfur world).

Hydrothermal vents run across tens of thousands of miles across the ocean floors, comprising billions of square miles. Given the (minimum) window for life's emergence of approximately 150 million years, organic compounds could be produced in vast quantities.



### **Günter Wächtershäuser's hypothesis**

Günter Wächtershäuser (a close friend of Karl Popper) has suggested a hypothesis on the origins of life, using the catalytic properties of various minerals, such as iron (Fe), nickel (Ni) and sulfur (S). These minerals are all found in abundance near hydrothermal vents. As we have seen, minerals may form an energy rich surface that can catalyze many reactions for the synthesis and assembly of monomers (and, as we will see, polymers) that may otherwise be infeasible. Wächtershäuser's hypothesis is unusually detailed, and has been designed to be rigorously testable and falsifiable. He has produced more than a hundred pages of specific chemical reactions that could lead to the first cyclical metabolic system. The core of the hypothesis is that metabolism can proceed without catalysts such as proteins, which are essential to metabolism in modern life, when the necessary organic components are in the presence of certain minerals (such as Fe, Ni, and S). He makes a number of core assumptions based on his observations. First, it is assumed that basic random pre-biotic synthesis as in the Miller experiment did not play an essential role. Wächtershäuser bases this assumption on a number of observations. First of all, the organic soup that would result from the Urey-Miller process would be far too dilute for the most interesting processes to take place. Second, the Urey Miller experiment catalyzed a large number of "molecular species" (group of molecules with similar properties) that could have played no conceivable role in life's origins. In this view, the Urey-Miller experiment is largely irrelevant to the origins of life. Second, it is proposed that life is not heterotrophic (that is, gathering molecules from the environment as food), but rather autotrophic (making its own molecules). Since the organic soup was probably much too diluted, he argues, it was an unreliable food source, and so life must have been capable of producing its own necessary building blocks. Third, it is assumed that energy did not come from UV radiation from the sun, or electricity, but rather from chemical interactions. It is argued that photosynthesis, which is the modern process by which plants capture the energy from sunlight, is far too complex to have been a prime source of food. Additionally, UV radiation and electricity are far too disruptive for most interesting chemicals to be stable. Finally, most life today uses chemical energy as a primary source of

energy. This process can be mimicked by organic compounds accumulating on unstable mineral surfaces. These surfaces release energy when they interact with other compounds, and thereby provide a stable source of energy which is much like the kind used by cells today. In fact, many of the key proteins which catalyze modern metabolism have, at their core, a cluster of Fe or Ni atoms. Finally, Wächtershäuser argues for a metabolism-first point of view. In this view, the elements of life arose not in the form of self-reproducing genetic material, but as a self-replicating metabolic cycle of chemical reactions. In other words, an early atmosphere, consisting of mainly  $H_2$  (hydrogen) and  $CO_2$  (carbon dioxide), under the influence of energy released by minerals, ultimately lead to the chemical elements that drive life. In his view, life was both inevitable, and would have arisen rapidly on the early Earth. We will return to Wächtershäuser's model when examining the origins of self-replication. His model has been duped "the iron-sulfur world".

### **Crystals**

Gustav Arrhenius has been one of the first people to suggest that certain crystal minerals may have played a major role in the synthesis of organic components. He was primarily interested in the common double-layer hydroxides, which may contain many different elements, such as Fe, Mg (magnesium), Cr (chromium), Ca (calcium), Al (aluminum), Ni, etc., in many compositions, but always in a two layer structure with a space in between layers (see figure 16). These spaces can be occupied by small molecules like  $CO_2$  and  $H_2O$ . The organic molecules become concentrated between the layers, and it has been shown that they have a tendency to form larger molecules that may not otherwise emerge from a primordial soup. By changing the composition of these crystal structures, they can be fine-tuned to perform various specific tasks. For instance, Arrhenius et al. managed to spontaneously synthesize sugar phosphates, which both form the backbones of RNA and DNA, and are a key ingredient for ATP, one of the most important molecules used in cell metabolism.

### **Zeolites**

Joseph Smith proposes that a diverse class of minerals called Zeolites may have played an important

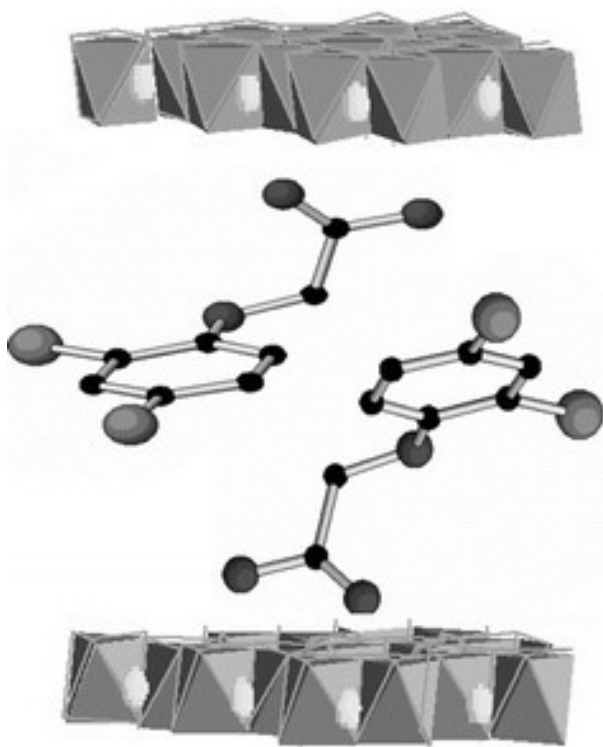


Figure 16: Double layer structure of 2,4-D in [Li-Al-Cl] LDH, with small biomolecules in between (image from <http://www.rsc.org/>).

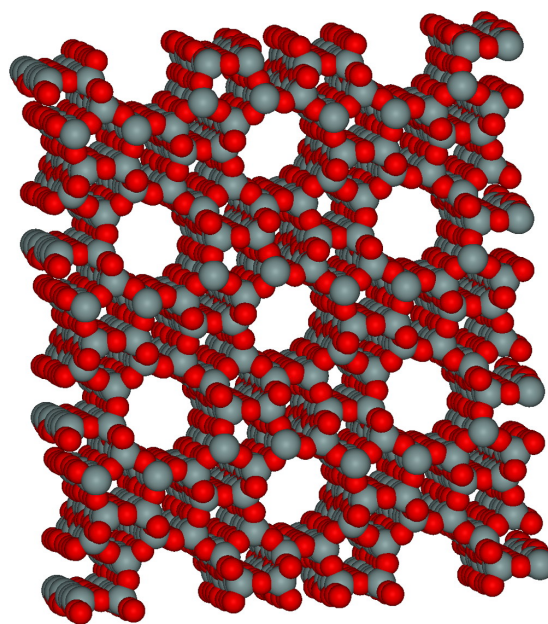


Figure 17: Zeolite lattice structure (image from <http://www.healthclinic.net.au/>).

role in the origins of biochemical elements. Zeolites have a lattice-like framework of small pores made up of silicon (Si), aluminum, and oxygen atoms (a typical structure is shown in figure 17). These canals are just the right size for a variety of simple organic molecules such as  $H_2O$  or  $CO_2$  to enter, while larger molecules are excluded. These molecules can then react inside the pores, and form larger organic components. Furthermore, under the influence of specific minerals, the larger molecules that land on the zeolite surface can be split into smaller equal size fragments (a trick commonly used in petroleum refinement), which can then be used in the construction of new, larger molecules. Zeolites are common in volcanic environments. Smith suggests that the canals inside these minerals may even have functioned as the first cell walls. So far, however, no experiments have been conducted to confirm this hypothesis.

#### **Molten rock**

Friedmann Freund et al. proposed that molten igneous rock may serve as yet another prime source

of organic components. Molten rock is over 1000C, and it inevitably contains mineral impurities, containing, for example, traces of  $H_2O$ , N and  $CO_2$ . As the rocks cool, different minerals will begin to crystallize in sequence at different temperatures. As they cool, the impurities in the minerals tend to accumulate on the outside of the crystal lattice, concentrated at defects in the crystal structure. These defects form elongated lattices that allow the now condensed compounds to bond with each other in a similarly elongated, chain like structure. Such a chain like structure is frequently seen in organic compounds, where different, smaller molecules are connected by a carbon backbone. The compounds are, of course, locked inside the surrounding rock, but Freund suggests that erosion eventually releases these elements. In principle, every mineral has the potential to drive a similar process, and given the enormous amounts of rock on Earth, similarly enormous amounts of organic components could be released. This mechanism could rival the Urey-Miller process in productivity (for instance, natural perito was found to contain  $\frac{100}{1000000}$  parts carbon, much of which was part of a carbon backbone). One problem with the hypothesis is that the destructiveness of these high temperatures may still be a significant problem for the formation of polymer chains. Furthermore, although observations suggest that many crystallized molten rock does indeed contain many organic molecules, it is difficult to test, since contamination is a very likely problem to occur. Almost every rock face on Earth is covered in microbial life, or at least in the remains of it, and is therefore contaminated with organic molecules.

From this we can conclude that rocks and minerals are likely to play a key role in life's origins. They act as catalysts for certain chemical reactions that would otherwise be unlikely to occur, they provide energy to power the process, and as we will see, they can provide templates for the formation of polymer chains.

### 3.2.4 Monomers from the Deep Hot Biosphere

It has been suggested by Thomas Gold that life may have first arisen deep within the Earth's core. Gold had previously suggested (and before him

Russian researchers) that the Earth's mantle may be a primary source for biocarbons, which form one of the most important groups of biomolecules. These biocarbons, he claimed, could be the primary source of the Earth's petroleum deposits. He based this view mainly on the presence of helium (He) in petroleum. Helium is a very light gas which could not have come from the Earth's atmosphere, and so the source of petroleum, Gold argued, must have come from a subterranean source, rather than having come from surface microbes that had been buried and decomposed. The mixture of helium, minerals and hydrocarbons would permeate upward as it is lighter than the surrounding rock, and the hydrocarbons within it would then be processed by ancient subterranean microbial lifeforms. This then produces the biofilm that we observe in petroleum that lead us to conclude that petroleum is organic in origin. Under Gold's controversial hypothesis, these hydrocarbons constitute a better, continually replenished food source than the primordial soup, or a puddle of condensed organic materials, which are, at least in principle, much more easily exhaustible, and could therefore lead to extinction. Similar to the deep ocean vent hypotheses, the basic monomeric compounds would be formed under the intense pressure and heat found in the Earth's deep crust, and Gold proposes that this was where the first proto-life may have formed. This explanation is lacking in detail, but many of the details can be borrowed from alternative hypotheses, such as the hydrothermal vents hypotheses and the various roles played by mineral surfaces as described above. It is also consistent with the observation that hydrothermal vent environments may promote reactions that result in products commonly found in petroleum, and that monomers may have been present in abundance during the formation of the solar system, which will be described below. Future discovery of microbial life below the surface of another planet in our solar system would also significantly increase the credibility of this theory.

### 3.2.5 Monomers from Space

One of the most surprising sources of monomers (and possibly even some polymers) comes from space. It has been known for some time now that space, particularly the huge nebulae which are the

birth places of star systems, contain large amounts of carbon (C), hydrogen (H), nitrogen (N) and oxygen (O). There is also evidence from spectral analysis in radio astronomy that these giant clouds of space dust contain large quantities of organic molecules. Over 140 different organic components have been identified in these clouds, some consisting of chains of at least 12 carbon atoms (and various other elements). This may be somewhat surprising, since outer space is bone-chillingly cold. The explanation for this process is that, as frozen mineral dust particles that are covered in ice travel through these clouds, they tend to pick up atoms and molecules. These hitchhikers are then subjected to UV radiation, which makes them more reactive, and they then react with other atoms or molecules on the particle surface to form increasingly larger molecular structures. Complex diagrams have been constructed that depict the efficiency at which particles would pick up molecules, which show that there will be a gradual buildup of increasingly larger molecules. As mentioned, nebulae are the places in which star and planet formation takes place. Stars and planets essentially form out of the gas in these nebula. During the formation of the so-called proto-planetary disc, which eventually condenses to form the various planetary and asteroid bodies in a star system, there would be a steady influx of organic particles. As a result, the amount of organic molecules in that disc is more condensed than in the surrounding nebula. This condensation increases the rate of synthesis even more, resulting in even more complex biomolecular structures, and so organic compounds are thought to be a significant component of planet formation.

### 3.2.6 The radioactive beach

A final source of monomers that I will discuss here is the radioactive beach hypothesis, coined by Zachary Adam. Adam claims that the close proximity of the moon to the early Earth could have concentrated grains of heavier radioactive elements, such as uranium, at the high tidal mark on beaches. According to this hypothesis, these radioactive materials may have provided the energy source necessary to have driven the formation of organic molecules, from acetonitrile in the water. In addition, radioactive monazite can release soluble phosphate into the beach sand. Phosphate is an

important building block for organic molecules like phospholipids. Additionally, radioactive actinides could have driven the formation of organo-metallic complexes, which could have played an important role as catalysts for early life. Adam's hypothesis is confirmed by computer models from the field of astrobiology, which show that these radioactive materials could show the necessary self-sustaining nuclear reaction. Under this model, amino acids, sugars and phosphates can all be simultaneously produced.

### 3.2.7 Chirality

The first person to offer an explanation of life's chirality was Louis Pasteur. Pasteur noticed that polarized light can be created by passing normal light through certain crystals. This means that such crystals filter out light with different polarities, while allowing light with another polarity to pass through the structure unaffected. Potentially, this can cause the selective breakdown of D-amino acids or L-sugars. In deep space, rapidly rotating stars can also emit polarized light. Another partial explanation from physics comes from the weak nuclear force. Most forces in nature are symmetric, but the weak nuclear force is asymmetric. Beta-decay (the emission of electrons) is driven by the weak nuclear force, and the end product of this decay is polarized. This too may select for molecules of a particular handedness. The problem with this explanation, though, is that the bias is less than 1%, and so the effect is likely to be trivial. Similarly, favored chirals may be slightly more stable than their mirror image, but this effect is likewise very minute. There is frequently a preference for bonds between two molecules of the same chirality over those of differing chirality, because they tend to fit better together. This also happened in Pasteur's experiments. So, each molecule might be seen as a micro-environment that selects for others of the same chirality. In this case, it is possible that polymers *have to* maintain chiral purity in order to form. However, synthesis experiments seem to contradict this.

### Chiral selection on crystals

The solution may come in the form of certain common chiral mineral surfaces (such as in quartz), which do show a strong preference for similar chiral-

ity. The crystals on which biomolecules form may themselves form to be chiral, purely by chance (the formation of a seed crystal is called nucleation). Local chiral environments like this are found everywhere on Earth. Most minerals are not chiral (though many of the more common minerals are), but even non-chiral crystals often feature patterns of chiral surface structures. Every grain of sand could potentially provide a chiral surface. Again, the chirality of mineral surfaces tends to be 50/50 between the enantiomers, so we have to look not at the global scale, but at the local scale. Molecules that are synthesized (seeded) on such local chiral template surfaces will themselves turn out to be chiral. Once a simple self-replicating system has been established on one of these crystal surfaces or subsurfaces (in ways we will discuss later), rapid growth can ensue in which all other molecules are consumed as food, and the chirality of the molecules in the self-replicating system will quickly come to dominate the local environment. There may then at some point have been multiple different competing chiral systems, one of which came to dominate over the course of time by natural selection in competition for resources.

The process of looking for an environment that is able to separate different molecules of differing chiralities is referred to as “resolving a racemate”. Hazen et al. carried out such an experiment on chiral selection using a racemic (=50/50) mixture of a specific amino acid (called aspartic acid). In their experiment, they used the common mineral calcite ( $CaCO_3$ ), which is also found in seashells. Calcite has the desirable property that, aside from having different faces with corresponding differing chirality, it also has cleavage faces (along which the crystal breaks more easily), which have no preferred chirality. These cleavage faces should exhibit no selection preference, and thus serve as a baseline. A baseline is important here, because of the inevitability of contamination. By comparing the ratio between differing chiral biomolecules on each chiral crystal face to this baseline, excesses can be measured that should be independent of the contamination that was not washed away during sterilization. Using a double blind test, they found a difference of a few percent, with increased L-chiral domination of amino acids on the L-chiral crystal surfaces, R-chiral preference shown for the R-chiral surfaces, and no preference at all for the non-chiral

surfaces.

Looking slightly ahead, this gives a plausible scenario for the formation of chiral polymers; as chiral monomers line up on chiral surfaces, they undergo polymerization, resulting in homochiral polymers (in this case, proteins). This also has significant commercial applications, for instance, in medicine.

Recent work (in 2003) has pointed to the amino acid serine as being a possible instigator of homochirality in amino acids. Serine forms very strong bonds with other amino acids of the same chirality, resulting in an eight-molecule homochiral cluster. Other amino acids can form weak bonds with amino acids of the opposite chirality. It is not clear how left handed serine in particular became dominant, but the results do suggest a way for homochirality to be maintained, once formed.

### 3.2.8 Conclusions on monomers

As we have seen, there is an abundance of potential sources of monomers, which constitute the building blocks of life. No single source may have been dominant, but it is safe to say that monomers were easily manufactured and present in abundance on the early Earth. But this is only the first step. The main problem at this point is how and why certain specific monomers are selected, organized and assembled, rather than how monomers can come into existence. This will be the topic of the next section.

## 3.3 The generation of polymers from monomers and the origins of self-replication

The next question to tackle is the leap that needs to be made from relatively simple monomers to polymers. In this section we will address research on the question how things like the genetic code, the cell membrane, proteins, and even systems of interacting biomolecules that form metabolic cycles have arisen. I will start with the formation of some general theories for the origins of polymers, then move to cell membranes, and then move on to proteins, and subsequently to the origins of metabolic cycles and the genetic code. But first, I'll mention some more general characteristics of macromolecules and problems that need to be solved.

### 3.3.1 The construction of macromolecules

To start with, the main problem that we need to address is how macromolecules are assembled. As we've seen, polymerization can be difficult under a variety of circumstances, particularly when in the presence of water or too high an influx of energy. As we will see, emergent behavior in these often complex systems can give rise to unexpected results. Second, we need to address the question of why these molecules are selected as a subset of the possible polymers that could exist. At some point, there may have been many macromolecular varieties that currently play no role whatsoever in any living system. We have seen some hints of possible explanations, for example, when discussing zeolite crystals and chirality. It is worth investigating why life today uses only a handful of building blocks, resulting in only a handful of basic types of chemical reactions, almost all of which are carbon-based. Another feature of polymers that is worth noting in this context is their modularity. Most polymers (or systems thereof) are members of a small number of major families (proteins or nucleic acids, and lipid cell membranes). All of these are modular in design; they can be broken up into smaller molecules that are by themselves monomers, like amino acids (in proteins), sugars (which are composed of ring structures or chains with a carbon to hydrogen to oxygen ratio of 1:2:1, and are typically locked up in polymers of millions of molecules like cellulose or starch), lipids (fats and oils used in membranes, or energy storage and for various other tasks) and nucleotides (in DNA and RNA). A possible explanation for this modularity is that it is simply more economical to do so. By using the same building blocks for many tasks, components can be re-used and recycled, and synthesis of one type of building block can underlay many different processes. In the same way that the cost of building a house with individually designed bricks would be immensely high, so too could the use of many different complex types of reactions and building blocks be costly for life. In other words, life that makes use of such economic modular design may simply have outcompeted other possible early life by means of being more efficient. A general observation that we made in the last section is that the early oceans are thought to have been simply too dilute. This implies that the probability of just the right molecules

coming together purely by chance is simply too remote. The only logical answer to this is that life must have concentrated on some kind of surface, as is a typical solution for many chemistry issues where diluteness is a problem. This could be the surface of a crystal or a mineral, for instance, in a tidal pool where cycles of evaporation and flooding can continually replenish and concentrate the chemicals in question, at the ocean floor (near hydrothermal vents), on a particle in space, or perhaps at the boundary between the ocean surface and the air. In essence, any contact point between two distinct materials could do the trick. Finally, it may be possible for carbon to assemble its own private surface from the environment, which can then also be used as a template. We will see this later on when we discuss the PAH world and RNA, for example.

#### Impact macromolecules

One of the more exotic possible origins of macromolecules comes from research on comet, asteroid and meteor impacts. At first glance, it would seem likely that an impact of this sort would break up any complex macromolecules that could have formed. This seemingly sensible assumption was tested by Jennifer Blank, who conducted several high velocity impact experiments. Blank shot stainless steel capsules containing various organic components (five different amino acids and water) through various rocks and minerals at approximately 4000 miles per hour. This creates approximately 200000 atmospheres of pressure and creates temperatures up to 1000C (note the irony in her last name). Blank discovered that pairs of amino acids form peptide bonds in every single run at the expense of some other, smaller molecules (which evaporated). In other words, although the number of organic components is reduced, their diversity increases as the result of such impacts.

#### Polyphosphates

Another mechanism that may have driven polymerization may be found in the properties of polyphosphates, which are formed by polymerization of monophosphate ions ( $\text{PO}_4^{3-}$ ). Several mechanisms have been suggested that could drive this polymerization process. Polyphosphates can cause polymerization of amino acids into peptides, and are

key precursors in the synthesis of compounds like ATP, which we discussed earlier. One problem with this theory is that calcium reacts with soluble phosphates to form the insoluble apatite. This means that we are required to find a plausible mechanism to keep calcium ions away from the phosphates. As we will see later on, lipid vesicles may be one such mechanism. One interesting idea about the origins of phosphorus is that it may have been introduced on Earth by meteorites.

### 3.3.2 The clay world

Although some of the polymers may have formed as the result of impact events, there are other, less disruptive ways in which they can spontaneously assemble. In particular, mineral surfaces may have played a major role, acting as catalysts, attractors and scaffolds in the construction of complex molecules. We saw examples of this for monomer formation earlier. Here I will explore how a similar principle can apply to polymerization. One such hypotheses, which places particular emphasis on the scaffolding principle, stems from the use of clays. Clays are nutrient rich, and they have a very regular, layered atomic structure, made up of two types of layers (one tetrahedral, which can incorporate minerals such as Si and O, and one octahedral, which can incorporate, among others, Mg, Al, or Fe. These layers are stacked in different vertical sequences of two (tetrahedral/octahedral) or three (tetrahedral/octahedral/tetrahedral) layers, with spaces in between. Billions of such layers may be stacked on top of one another in alternating ways, and clays are found everywhere on Earth, resulting in an enormous overall surface area. The layers are quite strong, but the space between them are quite weak, which is basically what makes clay slippery. Foreign molecules may accumulate between these layers and react to form increasingly larger molecules. What is more, clays are often electrically charged, which allows them to attract, and bond with, such molecules. These clays may also catalyze reactions. Daily and seasonal cycles of heating and cooling may drive this process. As we will see later, clays may form scaffolds for RNA and proteins, and has even proposed to have been the first form of self-replicating system (the so-called clay life hypothesis). One problem for the clay theory is that, as polymers become longer, they be-

come more strongly bound to their scaffolding. In other words, it is not immediately clear how they can become “unstuck” from the clays they reside on. A solution to this problem is to incorporate tiny bits of clay *within* the first cell membranes as they form. We will read about cell membrane formation below. Jack Szostak tested this hypothetical possibility by mixing together finely powdered clays, RNA nucleotides (which were made hyperreactive by addition of a catalytic molecular group), and lipids. He found that the clay absorbs the nucleotides, and is enclosed by forming vesicles. The result are protobionts containing the catalytic clay with small RNA strands.

#### The Clay life hypothesis

The clay hypothesis was taken a giant step further by Graham Cairns-Smith, who proposed the so-called “clay life theory”. This is where things turn a little odd. He suggested that fine grained (silicate) clay crystals may have been the first self-replicating systems, not by virtue of RNA, but all by themselves. In this theory, there is no initial role for biomolecules, and the first lifeforms were not carbon-based. Evolution, then, started independent of organic molecules. Cairns-Smith’s reason behind this hypothesis is dependent on several observations. Incidentally, Richard Dawkins supports this controversial view. Cairns-Smith is particularly interested in the properties of kaolinite crystals, illustrated in figure 18. First of all, it seems nearly impossible to build macromolecules without minerals. Clays functioned as essential scaffolding on which the complex molecules can be built, much like the scaffolds that hold up an arc before the top stone is placed. This scaffolding was later lost when it became expendable, as more efficient replication systems like RNA and DNA took hold. Recall that we can see possible remnants of the importance that minerals might have played in the current-day role of clumps of minerals in enzymes. Second, clay crystal layers have a varying internal structure that distantly resembles that of an information carrying structure, much like, for instance, RNA. Specifically, there are three possibilities for the “alphabet” of clay minerals (much like the four letter alphabets of RNA and DNA). First of all, the composition and orientation of the layers plays a key role. Recall that layers of clay can alternate

in (two or three) layered structures with spaces in between. Two layer and three layer structures may alternate. Each layer also has a specific orientation which can fall along one of three equally distant angles. Secondly, there may be variation within layers, called twinning, in which a single layer contains mixed surface patches in all three orientations. Third, clays can have a quite complicated chemical composition. Although the crystal structure itself is very regular, the incorporated minerals (e.g.: Fe, Mg, Al) may differ in sequence. Subsequent sediment can build new layers on top of the old ones that have the same orientation, chemical composition and surface defects. Thus, one can say that a layer of clay can grow in this way. When layers flake off, and is redeposited elsewhere by wind or water currents, this process may repeat itself, establishing new clay “colonies”. As Cairns-Smith points out, this looks a lot like a primitive form of reproduction. Additionally, the more stable configurations will tend to win out over time, which leads us to conclude that clays can evolve. This theory makes a number of predictions. First of all, the crystal structures must be reproduced accurately enough for the term reproduction to have any meaning. Furthermore, they must be able to compete for resources as they grow, and dissolve others in the process. More stable, rapidly reproducing (as the result of an abundance of certain types of chemical resources) patterns should be favored over unstable ones or ones with lower reproductive rates, and eventually come to dominate. In principle, at least, these predictions are testable, but it is not known how we can synthesize clays or sequence their structure as we do with DNA. Furthermore, much about clays remains unknown. We don’t know how they work at the atomic scale, we have a limited amount of knowledge about their surface properties, their structure is complex and very variable, and very fine grained, which makes it hard to determine which bindings occur. The hypothesis that crystals can act as reproducing systems with transferable information was tested in 2007, by Kahr et al., using potassium hydrogen phthalate crystals. The crystals were examined for imperfections, and then cleaved and used as seeds to grow new crystals. The imperfections were indeed reproduced by the clay “offspring”, but with many additional imperfections. Because of these additional imperfections, Kahr concluded that the



Figure 18: Kaolinite crystal growth (image from <http://originoflife.net/>).

crystals did not achieve the copying accuracy necessary for faithful transfer of information to the next generation. Of course, we do not know at this point whether this is true for all types of clays and crystals. Should this particularly odd hypothesis still turn out to be correct, then the question remains how we finally arrived at modern, carbon-based life. At some point, clays must have passed on their structure to organic materials, by the process we described earlier, in which the clay works as a template. The resulting RNA, DNA or protein structure would have similar genetic information as the clay that was their “launch stage”. Of course, there is no guarantee that information is still usable after this change in medium from clay to nucleic acid.

### 3.3.3 The origins of cell membranes

Before we start, it is handy to give a bit of terminology at this point. The formation of cell membranes deals with so-called vesicles. Vesicles, or liposomes (see figure 20), are small “sacks” containing various substances surrounded by a lipid bilayer (=double fat-based) membrane. Similar, but single layered structures are called micelles. In modern cells, li-



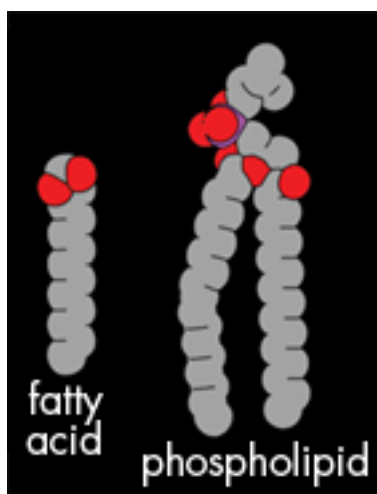


Figure 19: Fatty acid and phospholipid molecules (image from <http://exploringorigins.org/>).

posomes are used to transport or store various substances. When referring more generally to a collection of abiotically synthesized compounds that somehow self-organize, we may also refer to them as protobionts. Protobionts are thought to have formed spontaneously as precursors to modern cells (although a protobiont is by itself not considered alive). Coacervates (figure 21) are also protobionts; they are collections of macromolecules that assemble spontaneously when shaken in water. Finally, protobionts may consist of molecules that represent proteins, so called proteinoids, in which case they are referred to as microspheres. We will return to microspheres in the next section.

Recall that one of the most prolific problems faced in the construction of polymers is that they tend to break down when immersed in water. But here we have a paradox; almost all pre-biotic synthesis processes eventually wind up in the ocean, surrounded by water. And this makes sense, as modern cells too contain and are most often surrounded by water. At some point, then, life had to form a protective membrane to protect itself. The cell membrane in today's cells is comprised mainly of two opposing layers of lipids (of course, that's an oversimplification; modern membranes are vastly more complex, with many complicated structures in between that regulate cellular communication

and transportation of specific molecules, but we will not consider them at this point - it is assumed that these regulatory structures appeared much later in the evolution of life). Lipids are not fond of water. They are hydrophobic, meaning that they have a tendency to turn away from water when possible. So, a way must be found to make lipids compatible with cellular life. The answer to this problem comes from the addition of a phosphate group (a phosphate atom surrounded by four hydrogen atoms). Phosphate molecules are hydrophilic, meaning that they like water, and tend to face towards it when possible. A molecule that has both a hydrophobic end and a hydrophilic end is called an amphiphile. So, amphiphiles have a love/hate relationship with water. The molecule that is the result of adding a phosphate group to a pair of hydrocarbon chains is called a phospholipid (see figure 19), and it is these types of molecules that make up the membranes of modern cells. When amphiphilic molecules are immersed in water, they show a tendency to automatically arrange themselves to a state of lower energy. They line up end to end with the hydrophobic ends facing each other, and the hydrophilic ends facing out toward the surrounding water. When multiple such pairings of amphiphiles find each other, they line up side by side so that the inward facing hydrophobic ends are even more well-shielded from the water. When enough amphiphiles are lined up in this way they eventually tend to form a closed sphere, which is the state of lowest energy. This sphere, in the case of phospholipids, is called a lipid bilayer (see figure 20), for obvious reasons. It was discovered by Alec Bangham, who noticed that when lipids from egg-yolk were immersed in water, they spontaneously arrange themselves in this manner to form spherical vesicles. This lipid bilayer is used by all known cells. Figure 22 contrasts the lipid bilayer with a single layered spheroid called a micelle. Subsequent pre-biotic soup experiments on vesicles (by Luigi Luisi, among others) reveal that vesicles form quite easily. They show some interesting characteristics. Vesicles can grow by the incorporation of additional lipids. But perhaps more surprising, they are auto catalytic. That is, vesicles trigger the formation of other vesicles. Moreover, under the right conditions (the right acidity levels, and the right concentration of lipids), vesicles can divide into two new vesicles, which can then grow

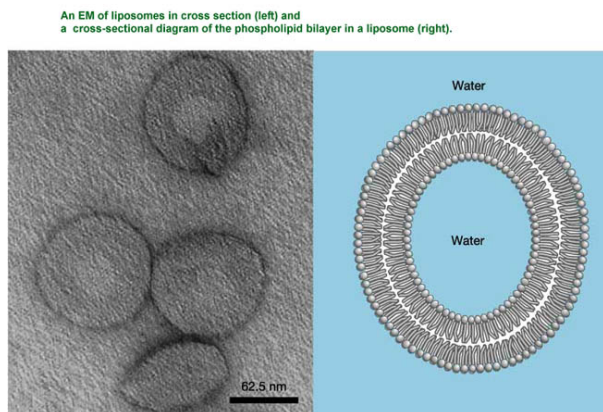


Figure 20: Liposomes and the lipid bilayer structure (image from <http://porpax.bio.miami.edu/>).

and divide again. This process looks surprisingly similar to cell division in modern prokaryotes - although it must be noted that it is obviously much simpler, and that vesicles are not typically considered alive by any standard. This leads us to the so-called “lipid world” hypothesis.

### The lipid world

The different large-scale scenarios for the formation of life on Earth are typically referred to as “worlds”. The lipid world, then, hypothesize that lipids played a central role in the formation of life on Earth. This scenario goes as follows. Lipids are created in abundance both on the Earth and in space. They end up in the pre-biotic soup where they self organize into vesicles. In the process, they capture primitive molecules (and possibly mineral particles like clays), which are typically thought of as information bearing molecules, such as RNA. If this RNA is capable of self replication, they can undergo simultaneous replication, leading, eventually, to the first forms of cellular life. This hypothesis was untested as of 2005, but progress is being made in this field to create the first synthetic life. One problem with lipid membranes is that the lipids are not well represented in the Urey-Miller experiments. A potential great source of lipids, however comes, again, from space. Deamer and Bangham were the first to propose such “self-organizing space lipids”. For vesicles to form, the lipids must have the right size and shape. For instance, if the lipids are larger

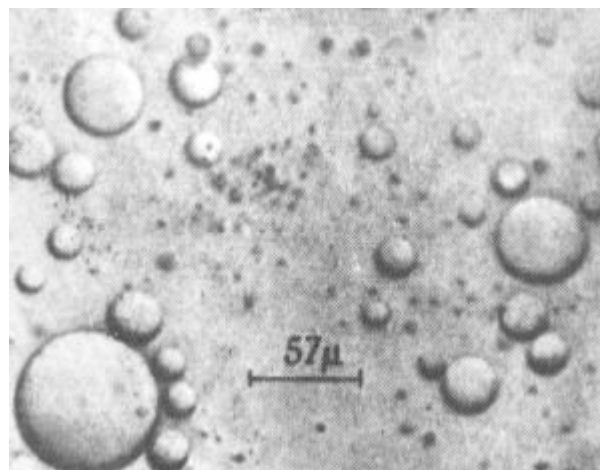


Figure 21: Coacervates (image from <http://www.daviddarling.info/>).

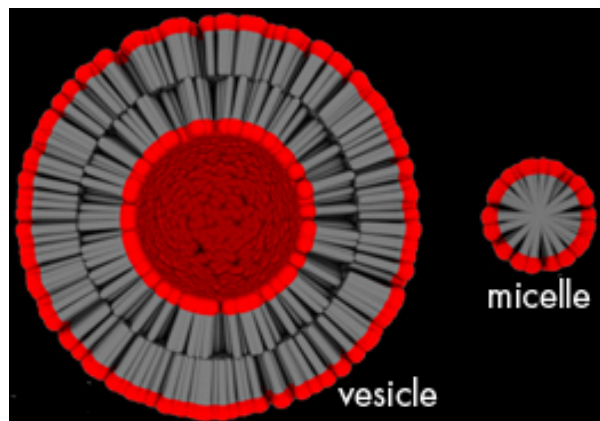


Figure 22: The difference between single layered micelles and bilayered vesicles (image from <http://exploringorigins.org/>).

than those found in current-day membranes, the concentration of vesicles is generally lower. To test the space lipid hypothesis, Deamer et al. examined carbon rich meteorites, particularly a so-called carbonaceous chondrite (see figure 23, a sample from the Murchison meteorite) in 1989. This examination revealed many biomolecules (about 3.5% of the total mass), including lipids. The experiment was set up as follows. The meteorite was broken down in a way to mimic weathering on the early Earth. The rock was ground down in a mixture of water, alcohol and chloroform, which was chosen because it does not affect minerals, but does dissolve a variety of biomolecules. Water and alcohol dissolve various amine acids and sugars, while chloroform dissolves various lipids. The resulting mixture was then centrifuged. This separates the denser materials from those less dense such that the most massive particles sink to the bottom, while the lighter elements remain at the top, resulting in a mineral/chloroform/water layering. It was found that the chloroform dissolved approximately 0.1% of the mass in the minerals, which indicates a high concentration of lipids (which was confirmed by chromatography). The solution of chloroform was then re-concentrated and the resulting concentrate placed in water. Vesicles were found to form readily. The test was repeated with multiple samples. These results were confirmed by simulations of space dust formation by Allamandera, who found in his results several so called PAHs (carbon ring structures which we will encounter again later when we discuss the origins of RNA, see figure 27), but also possible amphiphiles. When placed in water, these amphiphiles again formed vesicles. From this it can again be concluded that it is quite likely that our star system was likely to be rich in essential materials for life formation even before the Earth was formed. Additionally, the high pressure experiments on pyruvate conducted by Hazen et al. had also formed several potential lipid structures. Since pyruvate is a core molecule used in metabolism, this naturally leads to the idea that the formation of metabolism and the formation of lipids may be intimately connected. Specifically, pyruvate may provide a primitive metabolic pathway for the formation of lipids. By immersing the oily residue found during their experiment, it was found that vesicles did indeed form. This leads us to the conclusion that a simple reaction between water and

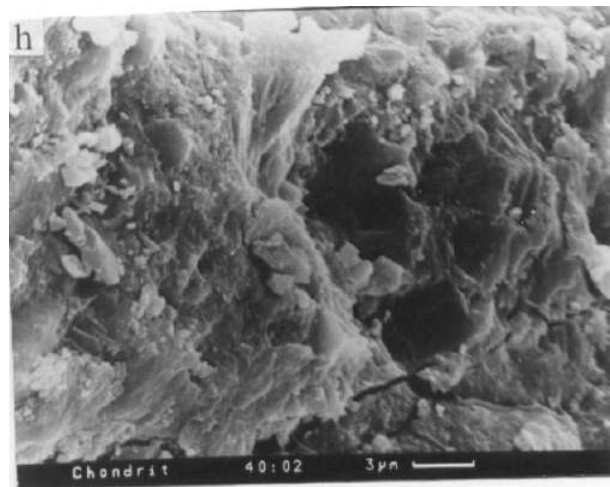


Figure 23: Carbonaceous chondrites in the Murchison meteorite (image from <http://www.panspermia.org/>).

pyruvate can eventually lead to the formation of lipids.

The lipid world comprises one of the best understood steps in the formation of life, but there were still some problems with the initial hypothesis. First of all, since modern cells use proteins to bring in food and export waste out of the cell, it is not clear how these primitive cells, having no proteins, would accomplish such a task. Second, bilayer formation is impeded by magnesium and calcium atoms, which are inevitably found in ocean water. Either the formation would have to take place in fresh water, or there would need to be an alternative pathway for the self organization of lipids. A possible solution to this second problem comes from Christopher Dobson et al., who proposed (in 2000) that lipids can organize in the atmosphere as ocean spray. Dobson noted that lipids can organize in another way from the spherical vesicles. They tend to accumulate on the surface, with the hydrophilic phosphate group facing towards the water, and the hydrophobic carbon chain tails facing towards the atmosphere. Waves can spray a mist of particles into the atmosphere. Each microscopic drop of water may contain lipids that will then form a single-layered sphere (a micelle) with the phosphates facing the water, enveloping the drop and any substances in it. The small droplets are quite

stable, and can drift in the atmosphere's currents for some time, or even months or years, reaching as high as the upper atmosphere where UV radiation is more intense, thereby promoting additional chemical reactions inside the droplet. Together, these aerosol vesicles constitute a vast number of micro experiments. When an airborne vesicle lands on the lipid covered surface, it will automatically form the secondary layer of the membrane, resulting in a bilayer enclosure. In this way, trillions of cell-like structures could have been present in the early oceans, which could be viewed as precursors of populations of cells. If this process occurred near a beach, where the tidal waves may have concentrated organic molecules (much like driftwood), this would increase the likelihood that the vesicles would contain many organic compounds. Since coastal waters are also generally warmer, evaporation may have further concentrated the organic soup. Vesicles composed mostly of water would tend to burst easily, but the presence of proteins or other amphiphilic compounds (such as PAHs) can increase the stability of the structure. If such compounds would increase the vesicle's integrity, then that vesicle would have a competitive advantage over others under natural selection. When a vesicle would burst (one possible primordial analog of reproduction), it would release the generated compounds into the environment, further increasing the availability of products that had accumulated within. Given time, this increases the chances of the appearance of the first self-reproducing cellular life form.

### 3.3.4 Self replicating systems

We are now ready to look at some of the models of self replicating systems. We already saw that a basic form of self-replication occurs in simple vesicles. However, although this is an interesting result, most people would be hard-pressed to call the vesicles by themselves living organisms. In the subsequent paragraphs, we will discuss several propositions for the origins of self-replicating systems. Most of these theories are divided into two camps; the metabolism-first view, and the genetics-first view. Although both approaches start out from a different viewpoint, they generally aim to adhere to three basic rules. First of all, there is a cycle of progression from smaller to increasingly

larger molecules. The largest of these molecules (either a DNA, RNA or protein strand, or the largest molecule in a metabolic cycle) should be able to split in two, thereby replicating the cycle. This allows for growth. Second, a plausible pre-biotic environment must be identified, which must supply all the necessary raw materials and energy, in a reasonably stable flow. The molecules that are part of the cycle must be stable enough to survive in this environment to partake in the next, duplicated cycle, to keep it going. In general, chemical energy is a preferred source over UV or electrical energy, because it is much more stable. Finally, an unbroken biochemical history must connect the Earth's past to its present. There must be a continuum along this path, and ancient "fossil" chemical pathways should be consistent with the model. Working from these premises, we will now look at some of the most important models currently in circulation.

### Autocatalysts

The basis of all the models of self-replicating systems is that they (the systems as a whole) must be self-sustaining, that is, self-catalyzing. Self-replication occurs when a molecule copies itself while consuming other, smaller molecules as food. The simplest such systems consist of one molecule, while more complex ones consist of a network of interacting component molecules. If the system only contains a single molecule, then that molecule must, by itself, be autocatalytic. It then acts as its own template to synthesize exact copies of itself. In order for such a single-step system to work, it must be self-complementary. That is, it must be equal to its own template. For instance, DNA is made up of complementary strands, but these strands are not necessarily self-complementary. Recall that we mentioned earlier that, in order for an RNA strand to be able to self replicate without any intermediate steps, it must be palindromic. This combination of properties is rare, but not non-existent. Dawkins writes about Julius Rebek Jr.'s findings, in which he and his colleagues combined amino adenosine and pentafluorophenyl ester with amino adenosine triacid ester (AATE), which is an autocatalyst. Rebek et al. managed to synthesize self-complementary, self-replicating molecules consisting of adenine, naptaline, and immite. The experiment demonstrated the possibility

that autocatalysts could compete within a population of molecules in which hereditary information was maintained from one generation to the next; a primitive form of natural selection. Unfortunately, this molecule is an unlikely organic precursor. Additionally, Reza Ghadiri has managed to synthesize self-replicating peptides (amino acid chains that are generally smaller than proteins). In 1996, Ghadiri reported a peptide consisting of 32 amino acids that managed to self-replicate. There was one small catch: these peptides had to have two distinct specialized reactive fragments of 15 and 17 amino acids respectively.

### **Cross-catalysts and autocatalytic networks**

Autocatalysis in single component systems is quite rare. There is only a small handful of molecules that have these properties. Complementarity to other molecules, by contrast, is much more common. Autocatalytic molecules also typically require a steady inflow of specialized chemicals. In addition, a self replicating system consisting of only one component is unlikely or unable to exhibit change from one generation to the next, and thereby evolution is inhibited. One solution to the first and last problems in particular can be found by means of cross-catalytic networks. Cross-catalysis is exhibited in a system of two (or more) molecules that can catalyze the formation of their counterparts. For instance, in a system where molecules AA and BB are present which are made up of fragments A and B respectively, and where AA catalyzes the formation of BB from many B's, and BB catalyzes the formation of AA from many A's, this system as a whole can self-replicate by cross-catalysis. Similarly, more elaborate systems may exist. We will see examples of this in Kauffman's hypothesis on metabolic networks, as well as Eigen's hypothesis of hypercycles in which genetic material is thought to play a central role.

### **3.3.5 The origins of metabolism**

I will now begin describing the metabolism-first perspective. Metabolism-first views are based on the observation that life inevitably requires a stable source of matter and energy to grow, survive, and eventually reproduce. It is argued, then, that such a reliable source had to be available before ge-

netic reproduction could take hold. By contrast, genetics first viewpoints maintain that life, to really be classified as such, relies on an ability to pass information to offspring. Genetic material, they argue, is the most likely way to copy this complexity from one generation to the next. And this view has a certain appeal for reasons of continuity; We know that this is the way current life does it. Metabolism without genetics, the argument goes, is just a series of chemical reactions without a direction or control. By contrast, metabolism-first proponents argue that life builds in small steps. Metabolic chemistry is far simpler than genetic chemistry. It requires only a relatively small number of relatively simple molecules for self-replicating behavior to emerge, as is seen in the reverse citric acid cycle, which is the basis of metabolism, as well as the starting point for life's biochemistry in all currently living cells. But of course, in order for a metabolism first view to be taken seriously, these small building steps must be specified and tested under realistic environmental circumstances. We will discuss genetics first views in a later section, but at this point it would be prudent to point out the paradoxical situation we seem to be facing; which came first? Or, perhaps, did the two arise simultaneously? Although a recent trend has been to explore this latter option, it is easier to imagine that these mechanisms came about in separate events. One of the main problems for any autocatalytic metabolic system is that there must be a way to avoid the many side reactions that may occur, which could disrupt the cycle. A model by Fernando and Rowe suggests that the encapsulation of the cycle within vesicles may be one way of avoiding this problem. A second problem that needs to be addressed is how metabolism may lead to the formation of nucleic acids (RNA and DNA).

### **Autocatalytic networks**

Stuart Kauffman noted that autocatalytic networks are the most likely form of proto-life. Such networks could possibly be vast, and have the potential to evolve by means of an increase in efficiency through the incorporation of more interacting agent molecules in the cycle. This generally leads to a number of nested cycles in which variability is nearly inevitable. According to Kauffman, such a network would most likely come in the form

of metabolic cycles, without the need for a genetic mechanism. Such a system would meet the minimum requirements for life: reproduction, growth, and evolvability. The problem with Kauffman's accounts is that they are purely hypothetical and very sketchy. No specific cycle is given, although Kauffman does mention a number of requirements. First of all, the system requires a reliable source of energy, preferentially in the form of chemical energy, which is more stable than other sources. Second, the system requires a reliable feedstock of organic molecules, preferentially common and small ones, like  $H_2O$ ,  $CO_2$  and  $NH_3$ . Third, metabolism requires that, unlike in the process of burning, energy is harnessed for the building of new organic component molecules, rather than dissipated.

### Origins of Proteins

Proteins are the current day workhorses of cells, and theories on proteins are therefore generally grouped with metabolism-first views. There are various hypotheses on the origins of proteins. Returning briefly to the clay world, Leslie Orgel proposed that proteins may have first formed on clays. When amino acids accumulate on clays, they polymerize to form small protein-like structures as they condense when water evaporates. These protein structures may be several dozen amino acids long. Orgel also noted that different minerals select different molecules from the solution to polymerize. He called this process "polymerization on the rocks".

### The proteinoid world

Sydney Fox proposed what he called the proteinoid world, in which proteins play a central role. Fox dried and baked amino acids on rock surfaces, using environmental conditions plausible for volcanic areas near tidal zones. These amino acids polymerized and formed protein-like structures which he called proteinoids. Fox observed that proteinoids can sometimes act as catalysts. They also sometimes organize into microspheres (see figure 24), with a bilayer structure. It should be noted that it is unlikely that these are the precursors to modern cell membranes, since modern membranes are composed of mostly lipids. The microspheres sometimes appear to grow and divide. Thus, this is a self-reproducing metabolic model, with interesting

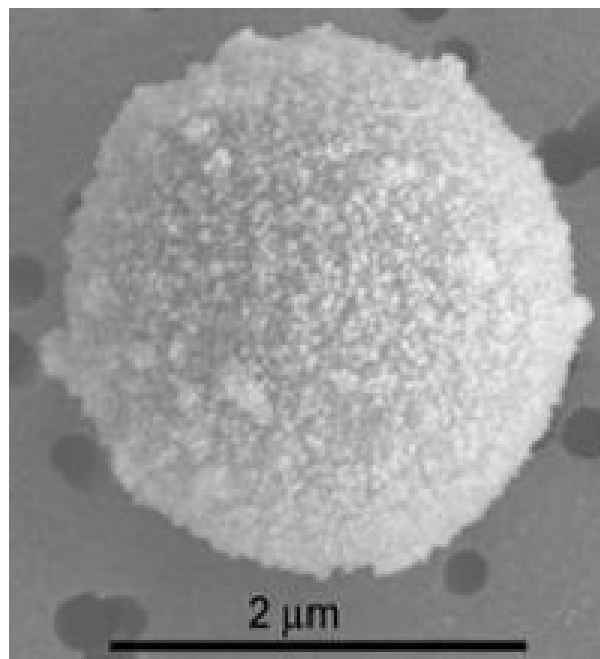


Figure 24: A proteinoid microsphere (image from <http://www.daviddarling.info/>).

behavior. Furthermore, since proteins play a central role in biology, this seems to form a viewpoint consistent with biological continuity. However, Fox has been the subject of skepticism. He has been known to make various far reaching claims, stating that the proteinoids were alive, that they solve the problems of life's origins, and even that they possess a rudimentary consciousness. This suggests that Fox may have lost his objectivity while studying these otherwise quite interesting structures. There is a more fundamental problem with a protein-first point of view, and indeed, it would seem, with any metabolism first viewpoint. The inevitable question, of course, is, provided proteinoids came before DNA and RNA, how could they "invent" genetic materials?

### The thioester world

Christian de Duve proposed a metabolic world called the thioester world. De Duve points out that, although his model is metabolism-first, he takes a neutral stance as to whether this means that metabolism preceded genetics as the first life. Ac-

According to his view, proto-metabolism came first, but life only arose with the inclusion of genetics within a cell. De Duve works from a plausible pre-biotic volcanic environment, where there are many mineralized and chemical components, specifically thioesters such as Acetyl-CoA. Thioesters are so classified on the basis of a carbon-sulfur thioester bond, which holds energy. Thioesters are crucial in metabolism today. They supply chemical energy, and form bonds with amino acids which make them more reactive, so that they can spontaneously assemble into short protein-like strands called multimers (which are smaller than polymers). De Duve hypothesized that thioesters played a crucial double role, both in providing a replacement for, and in the formation of ATP, which we mentioned earlier as a crucial primordial factor in modern metabolism. According to this hypothesis, an autocatalytic cycle may have emerged. De Duve's hypothesis is well aligned with Miller's results, and displays a clear continuity with modern biology. However, the details for this hypothesis are currently lacking. No specific molecular interactions are provided, and De Duve cannot give a concise answer as to which monomers triggered the synthesis of an RNA like molecule, and how they did so. The chemical reactions with thioesters that synthesize the monomers remained unspecified as of 2005.

### **The iron-sulfur world, bubbles and flat life**

I have briefly mentioned Gunther Wächtershäuser's model before. It is one of the most detailed hypotheses in origins of life theories, and has been designed specifically to be rigorously testable. His model, which uses a metabolism-first approach, is referred to as the iron-sulfur world. Wächtershäuser's model assumes that life is autotrophic. Chemical synthesis proceeds in small steps, by adding a few atoms at a time, using up small molecules such as  $H_2O$  and  $CO_2$  in the process. Contrast this with the Urey-Miller approach, in which life is heterotrophic, that is, in this view, life "eats" other, larger molecules already present in the environment, rather than synthesizing them from the ground up. This seems to be simpler; it is easier to just eat the available materials than to rebuild them yourself, and thus autotrophic cells have to be more complex than heterotrophic ones to allow for synthesis, or so the argument goes.

But Wächtershäuser argues that the true simplicity of his model lies in the reliance on small building blocks that must have been present in the environment in large quantities, using only a handful of such building blocks, and a small number of distinct kinds of chemical reactions in the process. Such a system, he argues, does not need to rely on chance resources in any given environment, but rather it makes its own food. One of the interesting characteristics, then, is that under Wächtershäuser's model, there is little or no role for the pre-biotic soup. The source of energy in the iron-sulfur world model is chemical in origin; it comes from minerals that are out of chemical equilibrium with their given environment, specifically pyrrhotite, which is a volcanic mineral with a one to one ratio of iron and sulfur. This mineral is often found around hydrothermal vents. It is unstable with respect to the surrounding sea water, and tends to transform into more stable minerals, in the process of which it releases chemical energy. When pyrrhotite ( $FeS$ ) reacts with hydrogen sulfite ( $H_2S$ ), it results in pyrite ( $FeS_2$ , which has instead a one to two ratio of iron to sulfur), plus hydrogen ( $H_2$ ) and energy release. But this is not the end of the reaction, because when  $H_2$  encounters  $CO_2$ , then, catalyzed by the energy released by the aforementioned reaction, this results in a formic acid molecule ( $HCOOH$ ). Note that here, the energy is not lost to the environment, but is used to catalyze new reactions, and this cascading of reactions is the point of the iron-sulfur model. The entire model is much to elaborate to discuss here in detail, but the key point to take away from it is that the reactions proposed by Wächtershäuser form a self-consistent network. So far, several steps in Wächtershäuser's model have been confirmed in the lab. The first step, which we described above, was confirmed by Wächtershäuser himself. Wolfgang Heinen and Annemarie Lauwers subsequently explored the model by immersing  $FeS$  and  $H_2S$  in water with a  $CO_2$  atmosphere. This produced a variety of interesting organic compounds, such as acetate, amino acids, pyruvate, as well as others. However, a cascade of reactions does not necessarily lead to the duplication of materials that is required for self-replication. For this, a closed metabolic cycle is required. Wächtershäuser is ultimately trying to accomplish this by synthesizing the reverse citric acid cycle. This cycle, then, is at the heart of the iron-sulfur world model. Re-

call that the closely related (normal) citric acid cycle is the core metabolism of every living cell, and this thus fits well with biochemical continuity. During this cycle, larger organic molecules made of carbon, hydrogen and oxygen are broken up into increasingly smaller fragments, while releasing energy. Recall also that the cycle can run in reverse. In this case, increasingly larger molecules are built at the cost of smaller ones (using energy or a catalyst). In current-day autotrophic life, this is how practically all essential biomolecules are built. The aforementioned experiments have shown that at least one of the key ingredients that drive the cycle (pyruvate) can be spontaneously synthesized under Wächtershäuser's model. However, in modern cells, the reverse acid cycle depends heavily on the use of complex enzymes to catalyze reactions that would otherwise not occur. And, perhaps as a consequence of this, no one has (as of 2005) reproduced the crucial step in the reverse citric acid cycle from pyruvate to (stable) oxaloacetate (because oxaloacetate always breaks down in water). So the question is then how this cycle got going without the use of such catalysts. Wächtershäuser proposes that iron sulfites can promote reactions in much the same way that modern enzymes do. He draws on the observation that many modern enzymes have iron, nickel or sulfur groups at their core that look exactly like small bits of sulfite minerals. Such non-enzymatic reactions would be less efficient than modern cell metabolism that relies on catalytic proteins, but since there was no competition at the time, even such a less efficient system could have flourished and dominated over other reactions by their self-replicating characteristic. Wächtershäuser notes that in many chemical reactions, among which many of those found in the citric acid cycle,  $H_2O$  can be substituted for by  $H_2S$ .  $H_2S$  is more reactive than water, and so reactions using  $H_2S$  may be more efficient. The formation of pyrrhotite near hydrothermal vents gives a clear indication that  $H_2S$  can be found here. As there would be less  $H_2S$  available, once the sulfite version of the cycle was established, it would only be a matter of time before the  $H_2O$  variant would be introduced, which, having a more reliable "food source", would then come to dominate. Wächtershäuser's arguments, then, make the prediction that the presence of  $H_2S$  leads to faster reactions and more available energy. Unfortunately,

this claim is not easy to test, as  $H_2S$  is very toxic, and chemicals formed in the reactions may be even more so. Hazen et al. have however performed experiments with citric acid in water under vent-type conditions (2000 atmospheres, 200C). They found indications that there may be not one, but rather two distinct pathways of cyclic reactions. The first, which corresponds to the normal reverse citric acid cycle, is duped the alpha pathway. The alpha pathway hits an unfortunate dead end, because oxaloacetate breaks down in water and can thus not be used in further reactions. However, there was also indication of some second, unknown pathway, called the beta-pathway, that is similar to the citric acid cycle, rather than the reverse citric acid cycle. It is speculated that this poorly understood beta pathway may once have been part of a now-extinct primordial metabolic cycle. Although it is still not well understood, it appears that at least some parts of the beta pathway can be reversed under the influence of NiS, which would have been present at vents, thereby establishing an alternative reverse cycle. Finally, one should note that the experiments thus far were conducted using water, rather than hydrogen sulfite, as Wächtershäuser proposed. It is important to note that the iron-sulfur world remains experimentally unsupported. Orgel, who we will encounter in the next section, believes that there is reason to suspect that this may remain so. An experiment performed by Wächtershäuser and Huber in 1998, yielded only a relatively small percentage (0.412.4%) of dipeptides, and an even smaller amount (0.003%) of tripeptides. Under the circumstances of the experiment, hydrolysis of the dipeptides occurred rapidly, and the criticism has been made that the experimental setup was lacking in organic molecules that could cause cross reactions and break the chain.

Using the iron sulfur model, three different scenarios have been proposed for the origins of life. The first and simplest is that lipid vesicles enveloped the first self-replicating metabolic cycle. The second scenario, proposed by Russell and Hall, is that iron-sulfite bubbles may have acted as the first cell membrane. Such bubbles form spontaneously at hydrothermal vents because the less acidic water emerging from the vents comes into contact with the more acidic ocean water. These bubbles, it is proposed, can act as membranes that enclose the metabolic chemicals. The energy, in



this scenario, comes from the contrast in acidity between the inside of the bubbles and their external environments. The third scenario, which was proposed by Wächtershäuser himself, comes in the form of “flat life”. In this scenario, the first self-replicating metabolic cycle appeared as a thin layer of reactants growing on a sulfite rich mineral surface, such as that found around hydrothermal vents. As this cycle self-replicated, it would grow to spread outward laterally as a thin coating. Pieces of this coating could then break off and attach to other rocks, where they would behave as cloned colonies of the original - the analog of reproduction. During this process, variations could arise due to the difference between the minerals and the environment, leading to several competing “species” of flat life. This primordial form of “life” may be much more resistant to high temperatures, and may therefore exist even today deep within the Earth’s crust, beyond the reach of more efficient modern life. This is off course hard to detect, and the claim is therefore commonly dismissed by biologists, who more commonly hold the genetics-first point of view, which will be discussed next.

One of the latest versions of this hypothesis was suggested by Martin and Russell, in 2002. According to them, cellular life may have formed inside the microcaverns of deep sea hydrothermal vents. This would solve several problems for the hypothesis. First of all, it would provide a means of concentrating newly formed molecules, which would increase the chances of polymerization. Secondly, the flow of hydrothermal water through the vent structure would provide a constant source of building blocks, as well as energy and catalysts. Third, the temperature declines rapidly when moving away from a vent, and this establishes an optimal zone for different reactions at different distances from the vents. For instance, monomers could form relatively close to the vents, with polymerization occurring in the cooler, more distant soil. Fourth, lipid membranes can form after all other cell functions have been developed, and then enclose this working metabolism. Finally, the model allows for a large number of subsequent steps in the development of early life to occur in a single structure, including monomer synthesis, protein and peptide synthesis, the synthesis of RNA, and finally even the development of DNA. The development of a lipid bilayer membrane may not have happened until quite a late stage in the

development of life. Under this model, then, the last common ancestor was located and developed inside the microcaverns of a hydrothermal vent system, rather than free-floating in the ocean. Life was only able to move outside of the vent system once the first cell membranes formed. Interestingly, cell membranes in archaea and bacteria, as well as eukaryotes, are quite distinct, in that completely different lipids are used in its formation. All other features of life seem to be similar in other aspects of physiology.

### 3.3.6 The origins of RNA

The main alternative to the metabolism first point-of-view is the genetics first point-of-view, the idea that life started with self replicating and autocatalytic genetic molecules, such as RNA, rather than with a metabolic cycle. Such a viewpoint has obvious appeal to biologists, who correctly observe that genetics is at the heart of modern life. Looking back at the clay world, one possible source for the origins of RNA may be polymerization on clay surfaces. James Ferris noted that certain clays (specifically: montmorillonite, see figure 25) can act as scaffolds for RNA. They activate the bonding between nucleotides when using a solution of so-called “activated” nucleotides and imidazole (activated nucleotides contain an extra molecular group that increases their reactivity). Without these clay minerals, nothing happened in Ferris’ experiments even after weeks of waiting, but when the clays are included, the nucleotides link up to strands of length 10 within hours, and within weeks, strands of 50 nucleotides were formed (recall also Szostak’s experiments, mentioned in the clay world discussion). Although these strands are random in nature, it is not hard to imagine that such random linking, given enough opportunity and time, could eventually lead to spontaneously self-replicating strands, and more. However, one major problem for any RNA-first view is that nucleotides, which are the basic building blocks of RNA, seem to be extraordinarily difficult to synthesize. A potential solution to this problem comes in the PAH world hypothesis, which will be described below.

### Hypercycles

In the early 1970s, Manfred Eigen et al. tried to investigate the transition from a chaotic primor-

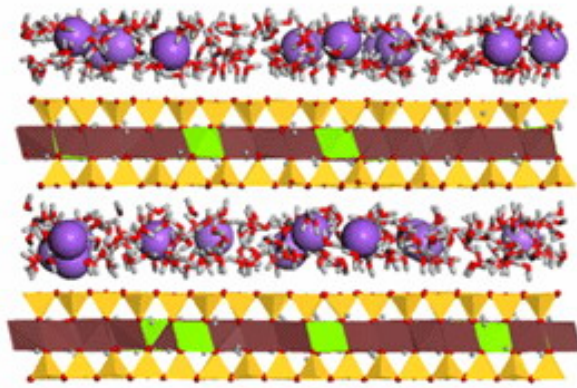


Figure 25: Calcium montmorillonite structure, here adsorbing Uranyl(VI) (image from <http://www.rsc.org/>).

dial soup to a self-catalytic macro-molecular self-reproducing cycle, duped a hypercycle. In a hypercycle, an information system like RNA produces an enzyme which helps in the catalysis of another information bearing molecule, which in turn produces another enzyme, and so on, until the last enzyme aids in the production of the first information bearing molecule. From a mathematical point-of-view (see also <http://pespmc1.vub.ac.be/>) hypercycles can create quasi-species capable of undergoing evolution by natural selection. A boost to this theory came from the discovery that RNA can sometimes form itself into ribozymes, RNA enzymes capable of catalyzing their own metabolic reactions. However, these reactions seem to be limited mostly to self-excisions, in which an RNA molecule becomes smaller after each replication. Some much rarer reactions can add small additions to the RNA, but these are incapable of coding for any useful protein. In addition, the hypothesis also suffers from the aforementioned problem that they require the existence of complex biochemicals such as nucleotides, which are not synthesized under the Urey-Miller type conditions that this research took as its premise. Hypercycles currently only exist in the form of computer simulations (see, for instance, <http://walter.deback.net/>, which simulates parasitism in a hypercycle driven RNA world).

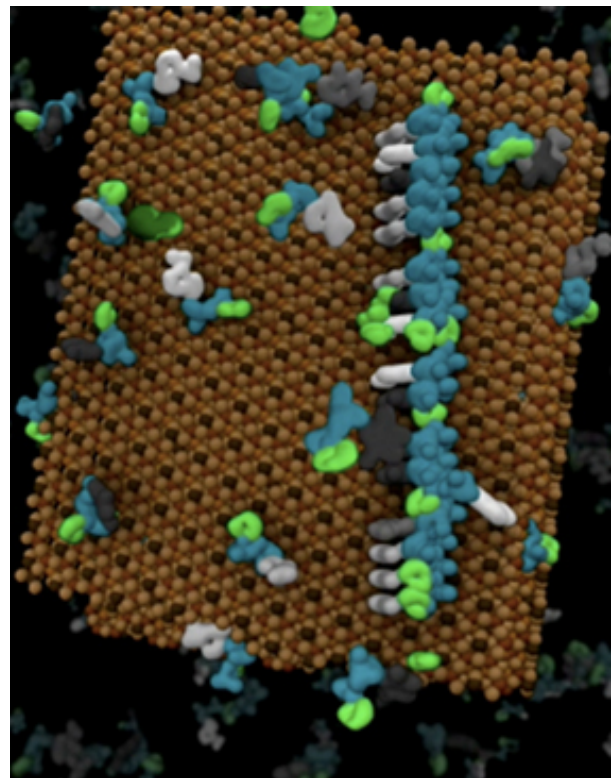


Figure 26: Nucleic acid polymerization on a mineral surface (image from <http://exploringorigins.org/>).

### The RNA world

The RNA world hypothesis is quite possibly the most influential model of life's origins at the present time. It was originally proposed by Leslie Orgel. The RNA-world hypothesis works from the observation that without RNA, there are no proteins, and without proteins, there is no metabolism (in modern cell life). Recall that a genetics-first system needs a stable molecule that is stable, self-replicating, and self replicating, and that there were four notable possibilities for the first information bearing molecules: self-replicating peptides (such as those in Fox's proteinoid world), DNA (requiring the simultaneous emergence of proteins and DNA), a clay world model (like that of Graham Cairns-Smith), or a nucleic acid like RNA. The RNA world, as the name suggests, takes the last of these to be the most likely candidate hypothesis. Underlying it are three basic assumptions:

1. RNA (or a molecule like it - see below) preceded DNA as an information storage system.
2. Ancient RNA replicated in the same way as modern RNA, by the matching of base pairs.
3. Ancient RNA played a catalytic role similar to that of modern proteins.

In addition, for reasons discussed below, it is commonly assumed that the most likely environment for this first RNA was within a lipid membrane (like a vesicle), and that metabolism emerged later as a means to make replication more efficient, leading to natural selection by a competitive advantage. The support for this model comes mainly in the form of the top-down evidence on the importance and central role of RNA in modern life, and the potential double role of RNA as both an information bearer and a catalyst, as discussed in section A, and in the previous two paragraphs. Additionally at least most of the component molecules of RNA can be synthesized readily by Urey-Miller type experiments, as well as under a variety of other plausible early-Earth conditions.

Jack Szostak, who is currently researching the possibility of synthesizing artificial organic life, managed to engineer an evolving replicase RNA molecule in the lab that can replicate parts of RNA up to 14 basepairs long. This RNA functions as both a code and a catalyst, and can serve as a copying template. He has also shown that certain catalytic RNAs can join smaller RNA sequences together, which, under the right circumstances, could lead to self-replication. Other functionalities could be added to such a self replicating molecule by mutations, such as copying errors, which could provide the raw material for natural selection to act on, thus opening the door to competition and evolution. The RNA world also makes use of (and is thus compatible with) the potential of vesicle or microsphere formation as possible enclosures in which RNA can replicate in a protected environment. One possible mechanism of replication is based on the self replication of RNA within a vesicle. No known geochemical environment could have supported an isolated, naked RNA strand, which would rapidly “starve” by a lack of nutrients in that environment or simply break down. Vesicles pose a possible solution to this problem, because the RNA could, in this way, find some protection

from the hazardous environment it would find itself in. Such “proto-cells” would grow as a result of the buildup of internal pressure by the replicating RNA. If we assume that the protocells are in close contact with other vesicles (possibly also containing their own RNA strands) with less internal pressure, this would promote lipid exchange between the membranes, resulting in growth of one cell at the cost of its neighbors. This results in a competition for space, in which protocells with more RNA would outcompete those with less RNA, thus selecting for an increase in strand complexity. Interestingly enough, this hypothesis has been tested by Szostak, who placed vesicles in a sucrose solution. The vesicles in such a solution do indeed grow by absorbing lipids from the membranes of neighboring cells. This does not happen when the cells are placed in an environment of pure water, but it does happen in the presence of (modern) RNA as well. A consequence of this is that RNA does not have to make its own lipids or guide a metabolism right away, and that self-replication can, at first, happen without metabolism, so that the first mechanism of selection would depend on the efficiency of self-replication. Related to this story is the work on evolving viral strains (of the QB virus) by Sol Spiegelman. In the QB virus, QB-replicase replicates QB-RNA, but it does so rather sloppily. By imposing strong artificial selection for rapidity of self replication by repeatedly moving the complete viral strands into a new medium after an increasingly short time span allowed for copying, Spiegelman was able to increase the copying time of the virus. After 74 trials, with average replication times decreasing at every trial, the total length of the virus was one sixth the original length, and replicated at 15 times the original rate. Spiegelman performed similar molecular evolution experiments for heat, acidity and a variety of other selection pressures, which we will not discuss here. In addition, one way in which these protocells could replicate provides an interesting insight into the problem of nutrient richness as well. As vesicles run through tiny pores in mineral surfaces, they are squeezed and stretched out, and they can possibly divide in this way, after which the new cell copies can grow. Recall that some such pores were found to have a size similar to that of modern day cells. This growth and division process happens much faster in the presence of fine clay minerals. Dur-

ing this process, some of these clay particles may end up inside the vesicles. This clay can then help assemble the RNA strands (as we have seen previously), so that these protocells would contain clay-bound RNA strands. The polymerization process is illustrated in figure 26. As mentioned, Szostak is currently trying to engineer artificial life in much the same way as described here. If this can be done, it would of course boost the credibility of the RNA world hypothesis. But, it should be noted that engineered life did not arise spontaneously and is therefore only considered weak evidence for the purposes of abiogenesis. As of 2008, no one has yet been able to synthesize a protocell in this manner (nor under more plausible more conditions).

In spite of all this, there are some problems with an RNA-first view of origins. First of all, RNA is a complex chemical element, and it relies on exact sequences of nucleotides which are much harder to synthesize than the simple chemicals needed for a metabolic system. Furthermore, RNA is several steps removed from the core metabolic cycle in modern cells. This is not a huge problem if we assume that life at first proceeded without metabolism, but the onion-layering around the current day core metabolism does seem to suggest that complexity was built around it, rather than the other way around. Second, it is not entirely clear where the RNA in question came from. RNA is hard to build, and no one has yet identified an experimentally tested and plausible mechanism to link individual nucleotides end to end in an RNA strand. If you recall Szostak and Ferris' clay-based experiments, in which the polymerization of RNA was accomplished by using clay crystals as a template, you'll note that these experiment relied on so-called activated nucleotides, which have an extra reactive chemical group attached to them which acts as a catalyst. The question remains, therefore, how these strands are synthesized. As we will see, a possible answer comes from the PAH world scenario for the pre-RNA world. This hypothesis also gives a possible answer to the another objection that is frequently raised: Although their components have been synthesized in several experiments, nucleotides, in their entirety have not yet been synthesized from scratch, despite several decades of effort. The main problem is that there is no single known plausible mechanism to build the necessary ribose backbone, bases and phosphate groups

in one single sweep, let alone bind the nucleotides together. The circumstances which favor the production of ribose are detrimental to the production of bases, and vice versa. By contrast, most of the elements necessary to establish a metabolic cycle such as the citric acid cycle are easy to synthesize. The question, then, is how to define a transition stage between simple chemicals and the production of self replicating RNA. The PAH-world hypothesis provides one possible answer. Furthermore, recent experiments suggest that the original size estimates for an RNA molecule capable of self-replication were severely underestimated.

Another way to look at this problem is reject the so called "naked gene" view, and adopt a metabolism first view, in which core metabolism is established first, followed by a simple information carrier that is more stable than RNA, followed by the more efficient RNA, which eventually came to dominate (until the appearance of the yet more efficient DNA). The metabolic world would have provided a more stable environment in which the RNA could form. Oparin's 1924 suggestion that self-replicating vesicles may have provided shelter from the elements is one of the first such proposals. As we've seen, the iron-sulfur world, the thioester world, hypercycles, and Kauffman's autocatalytic sets are others. So, even in a metabolism-first scenario, the RNA-world remains a critical stage in the origins of life, albeit a relatively late one, which came after the metabolic world, but before the DNA/protein world. Filling this gap, that is, understanding how we move from a metabolic world to an RNA world, remains one of the biggest open questions in origins of life research. One of the most essential steps is to solve the construction problem that RNA faces, which is part of the so-called pre-RNA world. We will discuss some possible, though highly speculative solutions in the next sections.

### **The pre-RNA world: Nucleotides and alternatives to RNA**

The synthesis of nucleotides, particularly uracyl and cytosine, has proven to be problematic. At 100C, cytosine has a half life of only 19 days (although this half life is 17000 years in ice). Furthermore, the generally accepted way in which ribose is synthesized, called the formose reaction, yields numerous sugars, while displaying no selectivity, as

Larralde et al. note, from which they conclude that ribose and other sugars are too unstable to have functioned as the first nucleotide backbones. The linkage between ribose and phosphoric acid in RNA (an ester linkage) is known to be prone to hydrolysis, a problem we encountered numerous times before. For much the same reasons, Miller has remarked that RNA itself is an unlikely candidate for the first genetic information carrying molecule, because of its instability and the problems with synthesizing nucleotides. Instead, Miller suggests that this first molecule may have been a precursor to modern RNA, which has the following properties:

1. It must be a long polymer (like RNA), with information carried as a sequence of similar molecules (much like RNA nucleotides).
2. This information is carried by the same nucleotide bases as used by modern RNA and DNA, namely adenine (A), cytosine (C), glycine (G) and thymine (T) or, more likely, uracyl (U).
3. Replication occurred by the same sort of base pairing mechanism as is used in the replication of RNA today.

Taken together, one can imagine this precursor of RNA pairing up during self-replications with nucleotides as found in a real RNA strand, once conditions became favorable, giving rise to the first true RNA. The most logical course of study, from this point of view, is to explore different backbone structures that can bind to the RNA bases A, C, G and U. Several interesting candidates have been found this way.

Albert Eschenmoser has theorized about more than a dozen backbone structures, resulting in at least seven new stable polymers. One such molecule is called TNA, for Threose Nucleic Acid. Threose is a simple sugar that is formed by the simple fusion of two carbon based molecules. TNA has not yet been synthesized experimentally, however, and most of Eschenmoser's backbones are no more stable than RNA under pre-biotic conditions. Another notable polymer, duped PNA, for Peptide Nucleic Acid, was synthesized by Peter Nielsen, using an amino acid backbone. PNA is appealing for this reason, because we know that amino acids would have been present in abundance on the early Earth. However, PNA has also not yet been synthesized under

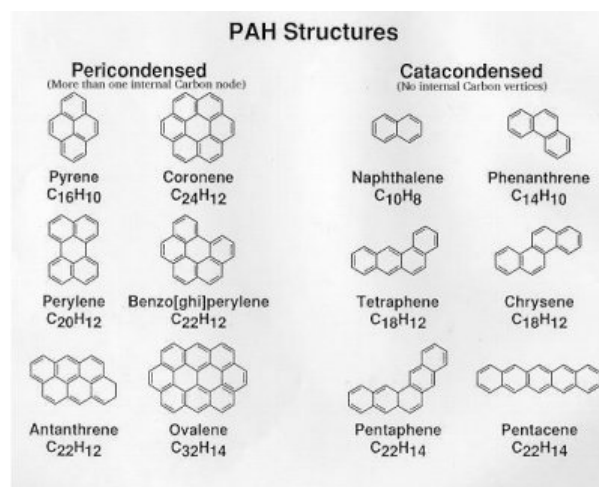


Figure 27: PAH structures (image from <http://www.daviddarling.info/>).

plausible early Earth conditions. Both TNA and GNA have no obvious way of emerging through self-organization. A final example, GNA, for Glycerol Nucleic Acid, were synthesized by Ueda et al. in 1971, which uses repeating glycerol units (containing only three carbon atoms) linked by phosphodiester bonds. This base pairing is much more stable than that of RNA or DNA, and a high temperature is required to melt a double strand of GNA. It is also the simplest of the known nucleic acids, thereby making it a strong competitor for the RNA precursor role. Although all of this remains speculative, the pursuit of alternative genetic molecules to RNA and DNA turns out to have commercial applications. New P/G/TNA-like molecules have been synthesized to interact with RNA and DNA without interfering with normal cell functioning.

As recently as January 2009, Tracey Lincoln et al. have managed to synthesize (artificially) the first RNA strand that can self replicate indefinitely. <http://www.scienceblog.com/>

### The pre-RNA world: The PAH world

Another potential solution to the nucleotide problem comes from Simon Nicholas Platts, who has duped his model the PAH world. Unlike the previously mentioned attempts, Platts has based his model on a logical model of self-assembly. As you

might imagine, it relies heavily on PAH molecules (see figure 27), Polycyclic Aromatic Hydrocarbons, which we encountered several times before. Recall that Allamandera found experimentally that PAHs are likely to be abundant in space, a hypothesis that is confirmed by the identification of multiple PAH-rich meteorites (specifically, Mars meteorites). Furthermore, in January 2004, it was reported by Witt et al. that spectral signatures of anthracene and pyrene had been found in the light emitted from the Red Rectangle nebula. Recall also that vesicles are more stable in the presence of PAHs. So far, there is no strong experimental evidence for this hypothesis, but its logic is plausible. The story goes as follows. PAHs are molecules that consist of multiple cyclic carbon molecules locked in a flat, regular grid (see, for instance, <http://www.tightrope.it/>). They are thought to have been abundant in the pre-biotic soup. PAHs are not usually easily soluble in water, but can, when exposed to sunlight, be chemically modified, so that their usual outward facing H atoms are lost, and can subsequently be replaced by OH groups. When this happens, their solubility is much increased. As noted in the section on lipid vesicles, the resulting molecules are amphiphilic molecules, that is, they have but a hydrophobic part (the carbon rings) and a hydrophilic part (the OH groups at the edges of the molecules). Because of this, they will self organize (for reasons similar to those that apply to pairs of phospholipids) in a stack when placed in water (see figure 28), so that the inner rings are shielded from the water, while the hydrophilic outer edges face the surrounding water. These edges are chemically reactive, and can bind to small, flat molecules like nucleotide bases, by means of a pair of OH bonds. As the stack of PAHs is not very stable, the PAH “plates” may swivel relative to each other. When this happens, any molecules that have bonded to the stack that aren’t flat are broken off, while flat molecules can remain attached. This means that there is a natural selection for nucleotide bases as the binding chemicals of choice. Furthermore, the bases themselves are amphiphilic, and will stack on top of each other in a similar way. When a stack of bases is lined up next to a stack of PAHs, they will react, and the result will be a stacking of PAH-base pairs. Interestingly, the space between the PAHs in a stack is 0.34nm, which is equal to the distance between bases in RNA and DNA. This

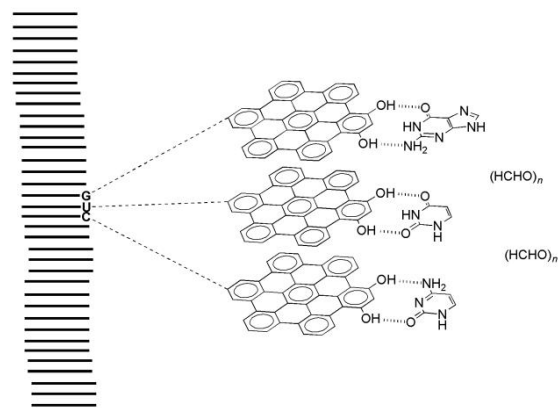


Figure 28: A stack of PAHs bonding to nucleotide bases(image from <http://tauceti.sfsu.edu/>).

is relevant, because the hypothesis is that a stack of PAH-base pairs, being more stable than a simple stack of bases, can sit in place long enough for other small molecules (possibly formaldehyde or amino acids) to bind to the other side of the bases to form a backbone, linking them together in a complete strand that is a true information bearing molecule. Finally, when the stacks float into a different environment (i.e. a different temperature or acidity), the strand could break away from the PAH stack and free float as a stable molecule. Potentially, such a molecule could fold back on itself and link up base pairs, which could allow it to act as a catalyst, and at some point, such a molecule might have also been able to self replicate. As you might imagine, this model is highly speculative, but it is considered a geochemically plausible, and conceptually simple path from the primordial soup up to a genetic world. The model makes many testable predictions. First of all, PAHs must of course stack in water, as expected. Furthermore, the PAHs in a single stack must be of about equal size and shape. Their edges must attract certain type of molecules, and the (flat) bases must be preferentially selected. All of this can, in principle, be experimentally verified. George Cody has pointed out that there is a large volume of literature on PAH self-organization into stacks of discs from coal research.

### 3.3.7 Exogenesis

A final set of hypothetical origins that I want to mention briefly are somewhat less commonly accepted than the hypotheses mentioned above, although not all of them are mutually exclusive. Specifically, I want to discuss exogenesis. Exogenesis is the hypothesis that primitive life may have originated elsewhere in (or even outside of) the solar system, on a nearby planet or in space. A closely related concept, called panspermia, holds that the seeds of life may be present all over the universe. Panspermia, however, is much less widely accepted as a realistic hypothesis, and there is no evidence to either support or falsify it. I will concentrate here on life in our solar system. One of the reasons why this prospect of extraterrestrial origins may be appealing to some is because of the short window for life's emergence; the most recent estimates from fossil research place the emergence of life at somewhere between 3.8 and 4 billion years ago, almost immediately after the Earth became habitable. As noted in before, organic compounds are relatively common in space. This is especially true in the outer solar system, where volatile compounds are not rapidly evaporated by sunlight. The Cassini-Huygens space probe has confirmed the existence of water and organic compounds in our solar system. Comets are encrusted by a layer of dark material that is thought to be composed of a tar-like substance, composed of organic material that formed from simple carbon molecules after exposure to ultraviolet radiation. Comet material raining down on the early Earth could have spread significant amounts of organic materials on its surface, and it is speculated that even very simple life may have formed in space and may have been brought down to our planet in the same way. Although there is only some very circumstantial evidence for this type of hypothesis, it extends the range of potential conditions under which life may have formed tremendously, from the early Earth's plausible conditions, to practically all conditions found in the known universe. Our current knowledge on extremophilic species suggests that life may be much more robust and versatile than once thought. A recent discovery of a bacterial ecosystem that derives its energy from radioactivity provides further support for the hypothesis. Jason Dworkin performed a recent experiment in which a frozen mixture of water,

methanol, ammonia and carbon monoxide was subjected to UV radiation, thereby providing plausible environmental conditions to simulate those found in outer space. The experiment yielded large quantities of biomolecules, which self organized into protobiont bubbles when immersed in water, of sizes measuring between 10 and 40 micrometers, which is a size similar to that of red blood cells. These bubbles seem to resemble cell membranes such as those found on Earth life. In addition, they fluoresced when exposed to UV light. This means that the more energetic UV light was absorbed and then re-emitted as lower energy, visible light. This was considered a possible way of providing the hypothetical primitive cell with energy, that could have been a precursor to primitive photosynthesis. Furthermore, it also acts as a sunscreen, by diffusing damage that would otherwise result from the UV exposure. This is also relevant for early Earth life, since the early Earth would lack an ozone layer, which currently blocks out the most harmful UV radiation. The ozone layer only emerged after photosynthetic life began to produce oxygen approximately 2.2 billion years ago. Of course, this type of explanation does not give us an actual explanation for life's origins (merely moving the origins location and conditions), but rather a broader set of tentative conditions that may be worth looking at. Under this scenario, life could really have formed anywhere in the universe, only to be subsequently dispersed, even to other star systems across the galaxy, by comet and meteor impacts. We may have to wait for samples to be gathered from comets and other planets (like Mars, see below) before we can make any more specific claims.

#### Life on Mars

One possible exogenesis scenario is that life originally originated on Mars, but was subsequently transported to Earth when parts of the Martian crust was thrown into space by a comet or asteroid impact. Mars is much smaller than the Earth, and therefore cooled faster than our planet did, allowing life to form more rapidly there, by a factor of hundreds of millions of years (which is significant compared to the 150 million year window that is considered the likely norm for Earth). It continued to cool rapidly after the supposed impact events, having now lost its atmosphere due to suffering

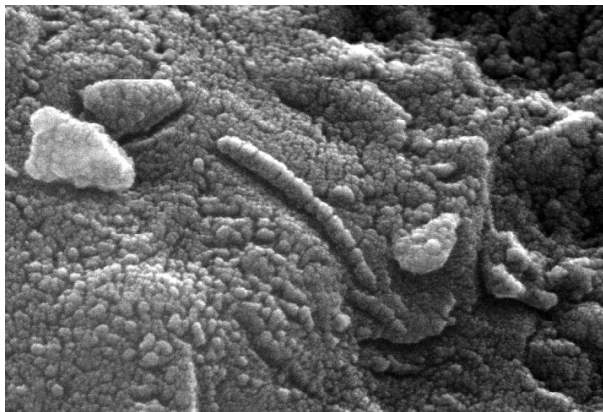


Figure 29: Mars meteorite ALH84001 sample (image from <http://tycho.bgsu.edu/>).

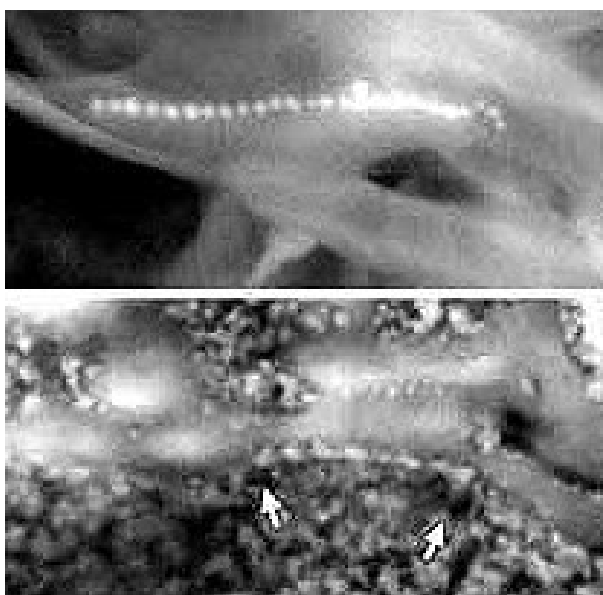


Figure 30: Above: modern magnetotactic bacteria showing chain of magnetite crystals. Below: chains of magnetite crystals in the Martian meteorite. Each crystal is about one-millionth of an inch in diameter. Image - NASA AMES (image from <http://www.abc.net.au/>).

from low volcanism, and it has by now become an inhospitable place for life. Nevertheless, NASA has been searching for signs of pre-biotic life on Mars. Mars meteorites have been found on Earth, and recent studies of meteorites found in Antarctica support the exogenesis idea. Allan Hills, who investigated a Mars meteorite, found large quantities of PAHs, which commonly form when cells are exposed to temperatures above boiling point. This points to presence of significant amounts of carbon on Mars. He also discovered microscopic globules of carbonate minerals, as are found in Earth cave walls. On Earth, these are often deposited by liquid water passing through cracks and fissures, which suggests that liquid water may have been present on Mars. This is also reminiscent of minerals deposited by microbes on Earth. Hills also found quantities of sulfite (in the form of pyritite) and ironoxide (in magnetite). These were ordered in an unusually pure and linearly ordered arrangement (see figure 30), which is only known from magnetotactic microbes, bacteria that use the Earth's magnetic field to orient themselves, to distinguish up from down (incidentally, when one places magnetotactic bacteria from the southern hemisphere in northern hemisphere soil, they will inadvertently bury themselves alive). Finally, Hills found various microscopic "sausage shaped objects" (see figure 29), that are somewhat reminiscent of known types of microbes, only far smaller. However, critics of Hills have since pointed out that none of his finds are conclusive. PAHs are common in the cosmos, and are synthesized by natural processes on both Mars and in interstellar dust. Additionally, the meteorites are very likely to have been polluted while sitting on the antarctic ice. In addition, carbonate globules can form by other processes, such as when minerals react with  $CO_2$ . There is some evidence that the globules formed above boiling point (though this is debatable), and so it may be unlikely that they could have formed by water running through cracks in the mineral, but even so, the evidence is not very strong. Thirdly, magnetites are common in meteorites, and although their arrangement is quite unusual, magnetotactic microbes require a sufficiently strong field to use magnetites for orientation purposes. Mars' magnetic field is much weaker than that of the Earth. Fourth, the "Mars fossils" were much smaller than any known microbe, consisting of no more than a few hundred



biomolecules. There are alternative known processes that could have produced similar structures. Finally, research has subsequently revealed that all known meteorites reveal signs of life. But this life is terrestrial in origin; contamination is nearly inevitable. Future Mars missions will have to reveal more about possible life once living (and perhaps still) on or within the Martian crust. It is of course possible that, if such life was found, it could have been transported from Earth to Mars, rather than the other way around. However, this is much less likely. First of all, Mars has a weaker gravitational field (Mars is about 1/10th the Earth's mass). Second, the Earth is closer to the sun, which means that the gravitational pull would tend to draw particles from Mars towards the Earth, but not the other way around. If a Mars rover did find life, then the possibility remains that this is due to contamination. The only true confirmation of extraterrestrial life, then, would come from the discovery of a so-called "second genesis"; the discovery that life emerged on another, distant planet that is almost certainly independent from the formation of life on Earth. A main sign of such life is to look for the presence of liquid water, and more importantly, an oxygen rich atmosphere. Alternatively, second genesis could be found on a more nearby planet if the lifeforms found differed significantly from all life currently found on Earth.

### 3.3.8 Multiple genesis

Not all scientists are convinced that life on Earth emerged only once. Different forms of life may have emerged nearly simultaneously (in terms of geological time scales). These other forms of life may either have gone extinct (perhaps having left distinctive fossils by their differing chemical composition, for instance, using arsenic instead of phosphorus). Alternatively they may currently live as undiscovered extremophiles, or be so similar to other life on Earth that they have simply escaped our attention. For example, Hartman notes that:

The first organisms were self-replicating iron-rich clays which fixed carbon dioxide into oxalic and other dicarboxylic acids. This system of replicating clays and their metabolic phenotype then evolved into the sulfide rich region of the hot spring

acquiring the ability to fix nitrogen. Finally phosphate was incorporated into the evolving system which allowed the synthesis of nucleotides and phospholipids. If biosynthesis recapitulates biopoesis, then the synthesis of amino acids preceded the synthesis of the purine and pyrimidine bases. Furthermore the polymerization of the amino acid thioesters into polypeptides preceded the directed polymerization of amino acid esters by polynucleotides.

Another reason why we may not be able to discern other primordial life comes from horizontal gene transfer between bacteria. As mentioned earlier, bacteria frequently exchange genetic material, making it nearly impossible to determine a straight path through the evolutionary tree. Many distinct organisms may in this way have contributed to what we now think of as the Last Universal Common Ancestor (LUCA) of modern life. Lynn Margulis' endosymbiosis theory also suggests that multiple bacteria and archaea may have entered into a symbiotic relationship to form the first eukaryotic cell. Such symbiosis is promoted by horizontal gene transfer, and this thus makes the relation likely to be even more complicated.

### 3.3.9 Early evolution

As we have already seen, the earliest form of evolution would act on many simple organic molecules on the early earth to sort them out, by means of attracting and concentrating them near a particular mineral. Some molecules are inherently unstable or unusually reactive, and would have been sorted out early. Others would have been too easily soluble. Their presence in the primordial soup would be too dilute, and eventually they would be removed from organic use for lack of reliability. Some molecules would bind permanently to surfaces of minerals that did not help them reproduce and take over, or would clump together into tar-like masses that would have been unable to react with anything else in order to form a self-replicating system, and would thus also have been no use for life. All of this selection may have been amplified by cyclical processes on earth (hot/cold, dry/wet, light/dark), such as UV fragmentation

of unstable elements over the course of days and seasons, the ocean tides, or the pulsation of hydrothermal water around deep sea vents. This pulsation would have delivered new chemicals into the system, which would then be absorbed by certain reactive minerals or eventually detached from their surfaces. This process would serve to concentrate a subset of molecular species. Over time, this led to a refinement of the available subset of chemicals in any given environment, each distinct environment having its own unique inhibiting and promoting factors. This finally led to a stable state of equilibrium, until self-replication could eventually emerge. After the first replicating system came into existence, evolution would take over. When part of a self-replicating cycle, even unstable chemicals could persist and increase in number if they could make copies of themselves more rapidly than they were being broken down. Self-replicating systems would initially thrive in competition with their non-reproducing neighboring chemicals, and eventually multiple replicants might meet, resulting in more serious competition. Eventually, variation in these complex cycles of replication would emerge, providing the raw material for natural selection to build on. Organic evolution would begin here. There would have been competition for resources and space, leading to an increase in complexity driven by a selection pressure towards increased efficiency and stability, eventually leading to the formation of the earliest life. The transition would have been fuzzy, but eventually prokaryotic life would have emerged, much like the bacteria and archaea that still exist today.

### The early evolution of DNA

It is commonly believed that DNA evolved from RNA. A system of proteins and DNA is more efficient than one of just RNA. An interesting observation for a possible transitional stage between RNA and DNA worlds comes from our genetic code itself (see figure 31). The most primitive and easiest to synthesize amino acids, glycine (GG\*), as well as alanine (GC\*), proline (CC\*) and arginine (CG\*), are all coded for by bases guanine and cytosine. Interestingly, both these amino acids and the bases that code for them are synthesized in Urey-Miller type experiments. These amino acids can self-organize to form functional proteins. Recall

|              |   | Second Letter                            |                                      |  |   |   |   |   |   |
|--------------|---|--|--------------------------------------|--|---|---|---|---|---|
|              |   | T  | C                                    | A  | G   |   |   |   |   |
| First Letter | T | TTT } Phe<br>TTC }<br>TTA } Leu<br>TTG } | TCT }<br>TCC } Ser<br>TCA }<br>TCG } | TAT } Tyr<br>TAC }<br>TAA } Stop<br>TAG } Stop | TGT } Cys<br>TGC }<br>TGA } Stop<br>TGG } Trp | T | C | A | G |
|              | C | CTT }<br>CTC } Leu<br>CTA }<br>CTG }     | CCT }<br>CCC } Pro<br>CCA }<br>CCG } | CAT } His<br>CAC }<br>CAA } Gln<br>CAG }       | CGT }<br>CGC } Arg<br>CGA }<br>CGG }          | T | C | A | G |
|              | A | ATT }<br>ATC } Ile<br>ATA }<br>ATG } Met | ACT }<br>ACC } Thr<br>ACA }<br>ACG } | AAT } Asn<br>AAC }<br>AAA } Lys<br>AAG }       | AGT } Ser<br>AGC }<br>AGA } Arg<br>AGG }      | T | C | A | G |
|              | G | GTT }<br>GTC } Val<br>GTA }<br>GTG }     | GCT }<br>GCC } Ala<br>GCA }<br>GCG } | GAT } Asp<br>GAC }<br>GAA } Glu<br>GAG }       | GGT }<br>GGC } Gly<br>GGA }<br>GGG }          | T | C | A | G |

Figure 31: The Genetic code (image from <http://plato.stanford.edu/>).

that tRNA is used to translate (mRNA from) DNA into proteins, so that the tRNA determines the genetic alphabet by mapping from triplets to specific amino acids. This mapping may be arbitrary, or it may simply be the most efficient way of translation due to chemical or physical restrictions. This suggests that a possible intermediate stage between the RNA and DNA worlds where the genetic code consisted of only two bases, C and G, and four corresponding amino acids. Later, this code may have become more complex as new nucleotides A and T/U were added. Since this more complex code can result in more complex and more efficient proteins, selection would favor this more complex code.

In a recent article (see also figures 32 and 33, and <http://www.sciencedaily.com/>) Bokov and Steinberg seem to have tackled the origins of the ribosome. This presents another milestone in our understanding of the origins of modern life and early evolution.

This is as far as I will take this document. The evolution of DNA and proteins is a subject that one can write a book about, and it takes us beyond the origins of life and into evolution. If you want to learn more about this subject, a good website to check out is <http://www.evolutionofdna.com/>. I

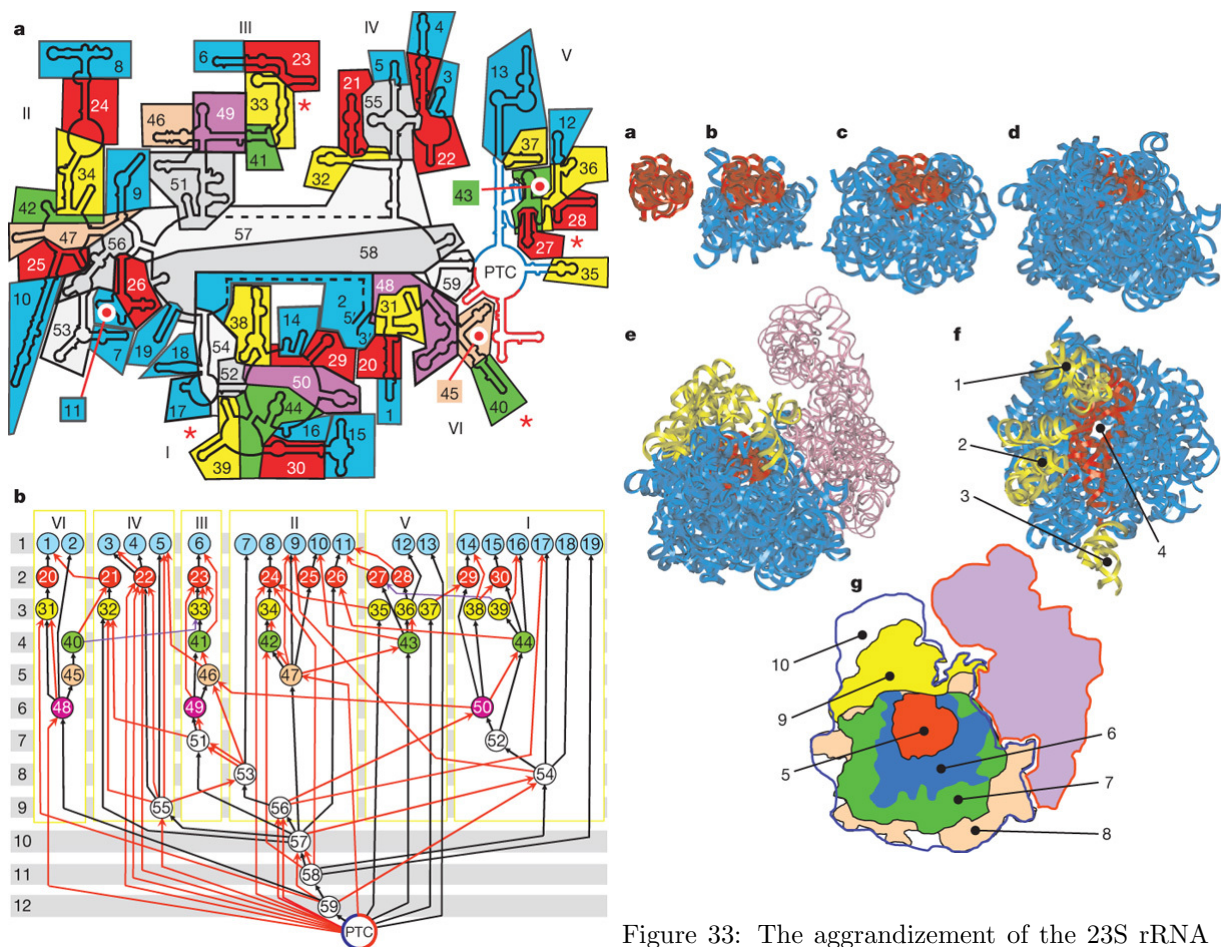


Figure 32: The location of the identified elements in the *E. coli* 23S rRNA secondary structure (a) and the network of D1 and D2 dependencies between them (b). Each element has the same colour in a and b. The roman numerals indicate secondary-structure domains. PTC stands for the symmetrical arrangement in domain V containing the peptidyl-transferase centre (the proto-ribosome). a, The two halves of the proto-ribosome are blue and red. Red asterisks indicate the four elements that form two non-local pseudoknots 2739 and 3340. b, An arrow connecting two elements  $Q \rightarrow P$  indicates that the position of P depends on the presence of Q. Black and coloured arrows represent D1 and D2 dependencies, respectively. Red arrows  $Q \rightarrow P$  represent A-minor interactions formed by a double helix of element Q and a nucleotide stack of element P. Two violet arrows originate from the dissection of two non-local pseudoknots (see Supplementary Notes 1). The numbers of levels are shown on the left. The detailed description of all elements and of all D2 dependencies is given in Supplementary Data 1 and 2.

Figure 33: The aggrandizement of the 23S rRNA structure during its evolution. ae, the proto-ribosome with 0 (a), 8 (b), 20 (c), 50 (d) and all 59 (e) elements added. The proto-ribosome is red, elements forming the proto-ribosome foundation are blue, the protuberances are yellow, and 16S rRNA is purple. The complete list of the elements forming structures ae is given in Supplementary figure f, The top view of the 23S rRNA structure shown in e. g, The positions of the parts of 23S rRNA shown in ae in the context of the whole ribosome. The structures of the 50S and 30S subunits are contoured by the blue and red line, respectively. 13 are the L7/L12, central and L1 protuberances, respectively; 4 is the exit channel; 59 are the structures shown in ae, respectively; 10 is the part of 50S subunit that does not include 23S rRNA. This part is formed by ribosomal proteins and 5S rRNA.

will conclude this section by simply listing a number of key events in subsequent early evolution:

1. The rise of prokaryotes and archaea.
2. The evolution of cyanobacteria, capable of undergoing photosynthesis, eventually resulting in the development of the current atmospheric composition of the earth.
3. The evolution of eukaryotes by means of endosymbiosis.
4. The evolution of multi-celled life, over a billion years ago (which is frequently illustrated using a current day organism called volvox, and its close relatives) and sexual reproduction.
5. From there, the very basic outline is: Flat worms → hemivertebrata → vertebrata → fish → amphibians → mammal-like reptiles → mammals → simians → hominids → human beings.

### 3.3.10 Conclusions

There is currently no single, broadly accepted scientific theory on the origins of life, but much headway has already been made in the last few decades. The models that exist can be broadly subdivided into three distinct categories:

1. Life began autotrophic, starting with metabolism, and only later incorporating genetic molecules into the mix.
2. Life began with genetics, possibly autotrophic, or possibly heterotrophic. Metabolism developed as the genetic material slowly became enriched.
3. Life began as cooperation between genetic material and a metabolic system. Although this may seem like a stretch, focus of research seems to be shifting towards this point of view, although no detailed hypothesis exists (as far as I'm aware).

The three stages that have to be solved to come up with a complete theory of the origins of life are summed up by Desmond Bernal:

- 1: The origin of biological monomers.

- 2: The origin of biological polymers.

- 3: The evolution from molecules to cell.

Bernal suggests that evolution by natural selection may have occurred as early as between stages 1 and 2. A rough outline of the general patterns that seem to emerge of the primordial soup of hypotheses that we've discussed can be given in the following way: First of all, as we have seen, monomers can be synthesized in a number of ways, including the primordial soup, deep sea vents, the deep crust, in mud puddles, or even in outer space. Secondly, it seems clear that the cell membrane forms by self organizing sets of phospholipids of an appropriate length into lipid bilayers. Third, several different hypotheses exist for the origins of the first self-replicating molecular system, be it an RNA precursor (or RNA itself), or a self-replicating metabolic cycle. An interesting development in late 2009 was published in [PNAS](#). Vasasa, Szathmrya and Santosa conducted a study in which they tested whether the stability of auto-catalytic sets is sufficient for such networks to have undergone evolution. If there is too much variance in how a network of molecules reproduces itself, then any effects of natural selection would be overwritten by the sheer rate of mutation. As with Kahr's results on clay crystal reproduction, their results suggest that such networks lack the required stability. What this means is that, although auto-catalytic sets may still have played a vital role in the origins of life, they would not by themselves have been capable of evolving, and would thus not be considered truly alive by the criteria that abiogenesis researchers generally agree upon. But the metabolic and genetic accounts are not necessarily mutually exclusive. Either way, at some time, RNA is thought to have dominated the globe. For instance, selection pressures favoring replication and metabolic efficiency may have first resulted in the development of ribozomes, and with them, the formation of small proteins. Eventually, this may have resulted in the first ribosome, which would have lead to more protein synthesis. Proteins are more efficient catalysts than ribozymes, and therefore become the dominant polymer molecules, leaving RNA to their modern use as mostly carriers of genetic information.

Much remains to be discovered in abiogenesis research, and undoubtedly, many new discoveries will be made. I'm sure some new ones have

been made that I myself am not aware of, as my knowledge is somewhat out of date. At any rate, I am optimistic about the future of this field of research.

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