The Origins of Life
And Precambrian Evolution (Ch. 14)

14.1 The RNA world.

- Until recently, the question of the origin of life was similar to the riddle of the chicken and the egg, which came first proteins or DNA? The dilemma this posed to biologists for many years can be seen in the idea of the Central Dogma first proposed by Francis Crick:

\[ \text{DNA} \rightarrow \text{transcription} \rightarrow \text{RNA} \rightarrow \text{translation} \rightarrow \text{Proteins} \]

DNA is the molecule that stores the necessary data to code for proteins, but proteins are necessary in order to transcribe and translate the DNA into proteins. So which came first, the DNA or the proteins?

- A clue came in the early 1980’s when scientists discovered an enzyme composed not of protein, but of nucleic acid, specifically RNA. These RNA enzymes are commonly called “ribozymes.” Dozens of naturally occurring ribozymes have been discovered. The phenotype of all these ribozymes (what the ribozyme actually dose) involves the formation and breaking of phosphoester bonds in DNA and RNA. This discovery was hugely important because ribozymes may be the key in solving the mystery of how life began. Scientists stopped asking which came first, DNA or proteins, and began asking, “Did life begin as an RNA world?”

- What other evidence is there that life may have began as an RNA world?

  - Ribosomal RNA (rRNA) found in all cells composed of a three dimensional structure of RNA and proteins that is fundamentally important in the translation process.

  - Transfer RNA (tRNA) are also three dimensional RNA structures necessary for protein synthesis.
Current evidence suggests that it is the RNA section of the ribosome, not the protein that actually carries out the catalytic steps in protein synthesis.

The basic currency for cellular energy is ribonucleoside triphosphates, such as ATP and GTP.

All these facts speak to the antiquity of RNA and to the possibility that RNA may have been both the genotype and phenotype in an ancient RNA, precellular world.

To test the hypothesis that life began as a precellular RNA world, scientists have attempted to evolve RNA in a test tube environment. Fig. 14.2 on page 472 of your text shows an experiment by Beaudry and Joyce (1992). The basic elements of the experiment were as follows:

1. The Tetrahymena ribozyme is mutated randomly. This molecule can cleave oligonucleotides of DNA and attach them to its 3’ end, but only at a very slow rate.
2. Only those ribozymes that can acquire a short tail of DNA, in specific time frames, are selected to “reproduce.” These tails are a primer for reverse transcriptase to copy the RNA into cDNA.
3. A second primer turns the cDNA into a double stranded DNA (dsDNA).
4. The second primer contains a promoter region for RNA polymerase to transcribe the dsDNA back into RNA, which can then cleave and bind to the free pieces of DNA primer.
5. After 10 generations the activity of the RNA pool towards cleaving DNA substrates had improved by a factor of 30.
6. Most importantly, changes noted in the phenotype (improvement in the rate of cleaving DNA) could be traced to mutations at four nucleotide positions in the ribozyme’s sequence.

Ribozymes demonstrated to do what might be the most fundamental test for whether or not something is alive: biological evolution.

- We now know that ribozymes can perform such functions as phosphorylation, aminoacyl transfer, peptide-bond formation and carbon-carbon bond formation.

- However, with experiments such as those conducted by Beaudry and Joyce, a protein was needed to “reproduce” the ribozyme. In an RNA world, there would not have been protein. In order for the RNA world to have greater plausibility, it must be demonstrated that RNA can copy itself i.e., “RNA-dependent RNA autoreplicase.”
• It could be argued that the point at which organic molecules such as RNA acquired the ability to self-replicate, life began.

• The hypothesis that an RNA molecule could replicate itself and act as a simple proto-organism is testable. If it could happen in a lab, it could happen in nature. Scientists are looking for a ribozyme that can catalyze the formation of a phosphoester bond. By starting with random sequences of RNA and selecting those that happen to be able to perform this function, scientists have begun to discover ribozymes that can perform this function. Figure 14.6 on page 476 shows an experiment by Bartel and Szostak that discovered ribozymes that can catalyze phosphoester bonds. Read the description on page 475.

• To date, however, no ribozyme has been found that is capable of self-replication. The evidence to date suggests it may simply be a matter of time.

14.2 How do we get to RNA?

• The likelihood of making RNA abiotically is too small. The consensus is that RNA probably evolved from a more primitive chemical system. In order to get to any self replicating chemistry (RNA or otherwise) the following questions need to be answered:
  • Biomolecules are made up of simple organic compounds. So where did these simple organics come from?
  • In order to construct larger molecules from simple organics, the conditions need to be favorable, and there needs to be an energy source. So under what conditions, and with what energy could these larger molecules have formed?
  • These larger molecules, or building blocks, must be able to self assemble into polymers such as RNA and polypeptides. How did this happen?
  • The environment can easily break down large biomolecules, so how were they protected in order to persist and evolve?

1) Where did the basic organic molecules come from?

• The biomolecules of life require a number of elements, including: carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus (in large amounts) as well as magnesium, calcium, and potassium in smaller amounts. These elements were present on early earth, but there atmospheric concentrations and chemical forms are not known.
• There are two possibilities for the origin of the basic building blocks, they were synthesized on earth, or seeded from outer space. The key unanswered question is whether or not the early atmosphere was oxidizing with high concentrations of O\textsubscript{2} and/or CO\textsubscript{2} or was it largely reducing with high concentrations of NH\textsubscript{3}, CH\textsubscript{4}, and H\textsubscript{2}.

• In 1953 Stanely Miller performed a famous experiment in which he circulated water vapor through a reducing atmosphere and past an electric spark. This turned the water a deep red. When he chemically examined the water he found amino acids had formed. Using similar experimental designs, other researchers have found a wide variety of biomolecular building blocks can form from this “soup” including amino acids, nucleotides, and sugars.

• It is unclear, however, if earth’s early atmosphere was as reducing as Miller assumed. It may have been dominated by CO\textsubscript{2} instead of CH\textsubscript{3} and N\textsubscript{2} instead of NH\textsubscript{3}. If the atmosphere were more oxidizing, then it would not be as conducive to the formation of life’s building blocks.

• The Oparine-Haldane Model. Oparine and Haldane were researchers who in the early part of the 20\textsuperscript{th} century that developed a hypothesis for how life began. It is often criticized because it depends upon liquid water for the formation of life, and it is unclear if the earth had liquid water when life arose. It is useful, however, as a null hypothesis upon which to test other hypotheses. The model is broken down into a series of steps assumed to have taken place in the waters or moist soils of early earth:

1. Nonbiological processes synthesized organic molecules such as amino acids and nucleotides.
2. These organic molecules are then assembled into polymers (peptides and oligonucleotides).
3. Some combinations of biological polymers become self-replicating and able to catalyze reactions. These polymers can then direct the formation of other biological structures such as cell membranes.

• The other possibility is that the building blocks of life were seeded from outer space in the form of meteors, comets and cometary dust. An example of this is the Murchison Meteorite. This meteor stuck the earth in Australia, in 1969. Scientists carefully collected the meteorite fragments, and when they examined the interior of the rocks they found the amino acids: glycine, alanine, glutamic acid, valine and proline. The fact that the amino acids were found in the rock’s interior and that the amino acids were racemic
(approximately equal numbers of D and L stereoisomers) were strong evidence that the amino acids were not contamination from life on earth, but were actually brought to earth on the meteor. This also demonstrated that amino acids could survive the heat of entering earth’s atmosphere.

2. Under what conditions, and with what energy could the building blocks of life have formed?

- As mentioned, in a reducing atmosphere, amino acids form rather easily. It has been demonstrated that purines also form easily under these conditions. Pyrimidines are not as easily formed abiotically, although some success has been made. Other researchers have demonstrated that under the correct environmental circumstances, ribose sugars can be derived from a cascade of condensations that begin only with formaldehyde.

- So it has been demonstrated that a good portion of life’s basic building blocks could be formed from plausible early earth atmospheres. But there are a series of problems that researchers must address with these abiotically produced molecules.

1. The existence of nucleotides and amino acids still leaves us a long way from RNA and proteins.
2. What is the origin of chirality e.g., why are the amino acids used in proteins only the L stereoisomer? Whether produced on earth or in meteors, both mirror images of the building blocks are made in roughly equal proportions. To make matters worse, polymerization of one stereoisomer would be inhibited by its mirror image (easily done when each stereoisomer would be in approximately equal numbers around the polymerizing chain).
3. In the case of sugar formation from cascade condensations, ribose constitutes only a very small amount of the sugar produced.
4. There are multiple ways a nitrogenous base could be attached to a ribose sugar, but only one is the “correct way” used by contemporary RNA.
5. Before a building block can be polymerized, each one needs to be chemically charged. Without energy concentrated within a membrane, how did these molecules attain this chemical energy?

Although ribozymes may solve the problem of which came first, proteins or DNA, the problem of where ribozymes came from is still formidable. It does appear possible that the early earth could have been rich in organic molecules and scientists are looking at various possible molecules that may have been precursors to the RNA world.
3. Assuming a prebiotic soup existed that contained the building blocks of life, how did these building blocks self assemble into polymers such as RNA?

- Biological polymers can be easily synthesized in water; however, they are broken down by hydrolysis as quickly as they are synthesized. It has long been doubted that polymers of sufficient length, which may act as a self-replicating primordial organism, could be formed under these conditions.

- Ferris et al. conducted an experiment in which a prebiotic soup containing activated nucleotides and the aluminum-silicate clay montmorillonite were mixed together. Montmorillonite was used because it is naturally occurring and organic molecules easily adhere to it. When the activated nucleotides adhered to the clay, the clay acted as a catalyst, joining the nucleotides together into a growing polynucleotide. Due to the fact that the growing polymer was attached to the clay, polymerization occurred more rapidly than did hydrolysis. This method produced polymers of 8-10 nucleotides in length. By using similar methods with differing clays, Ferris et al. has documented the formation of polynucleotides of up to 55 nucleotides in length. These experiments may demonstrate the possibility that a self-replicating primordial organism could have formed from a prebiotic soup splashing over minerals in sediments.

However life originally formed, we know that once conditions on earth were favorable, life formed relatively quickly. Researchers have estimated that the last impact events that would have been large enough to vaporize the earth’s oceans probably occurred between 4.4 and 3.8 bya. There is evidence that life was established by 3.85 bya and prokaryotic life was abundant by 3.5 bya. It is often said in the public arena, that the probability of life forming from abiotic processes is too great to have happened. Evidence for very primitive life occurring shortly after conditions were favorable argues against this. Also, the earth represents only one data point. In order to make any statements on the probability of life’s beginning, we need to examine many other worlds that have at least the basic chemistry from which life could evolve. Only then can we begin to determine the probability that life will evolve from non-life.

14.3 When Life Went Cellular

- As we have seen, some researchers are attempting to answer the question of how and when life evolved by determining logical ways in which inorganic and organic molecules could randomly combine to form self-replicating bio-molecules. Other researchers are attempting to answer the question of how and when life started by examining extant organisms, and attempting to build a phylogeny of all living things. By doing so researchers hope to determine what the cenanchestor (or cenancestors), the
common ancestor or ancestors of all living things, may have looked liked and when it lived.

- Of course going from a self-replicating bio-molecule to the cenancestor(s) is a big step. Life would have had to go from RNA to a DNA world. Very importantly, life would have had to develop cellular membranes. Researchers have found that certain mixtures of polyamino acids in water spontaneously form microspheres that are reminiscent of cell membranes. Perhaps cellular life began by a simple, process of a ribozymes being randomly trapped inside primitive microsheres. Once this process occurred, there may have been strong selection favoring these proto-cells. The membrane would have acted to protect polymers from being broken down, and would have allowed the concentration of chemicals such as activated nucleotides in much higher amounts than in free solution. It also kept the genotype and phenotype linked, especially as the phenotype became the domain of proteins. There would be no evolutionary advantage to a genotype, if its phenotype diffused away.

- Because of the geological processes that have continuously reworked the earth’s crust since it first began to form around 4.0 bya (or slightly earlier), not much remains of the fossil record for very early life. The oldest fossils that have been unambiguously determined to be true fossils are approximately 3.5 billion years old. These fossils, from a rock formation called the Apex chert in Western Australia, may have been primitive cyanobacteria. If this were the case, then life’s most recent common ancestor would have existed much earlier. This assumption is made due to the fact that cyanobacteria is presumed to be relatively complex compared to what the hypothetical cenancestor(s) would have been. The Apex chert fossils are interesting, but not very useful in attempting to build a phylogeny for all life. This is due to the fact that the fossil record going back from 2.5 bya is very spotty. We don’t know if the Apex chert fossils represent a link in the living evolutionary tree, or an evolutionary dead end.

- Until recently, attempting to estimate the phylogeny of all living things was all but impossible. As you learned, many popular species concepts have depended upon examination of the phenotype. Traditionally, this has also been the case in determining the phylogeny of a group. This methodology is difficult at best when working with prokaryotes that lack the structural diversity to allow the construction of morphologically based evolutionary trees.

- The last 25 years has seen a revolution in our ability to read nucleotide sequences in DNA and RNA. This ability allows scientists to build phylogenetic trees with characters based upon individual nucleotides in addition to the more traditional morphological characters. Even with the
use of molecular data, building the phylogeny for all living organisms has some very unique problems. Animals, plants and bacteria etc. are obviously very different. A gene must be found that performs the same function in each group. The gene must have enough sequence similarity between these very different groups to allow the construction of a family tree. (If the sequences are extremely different between groups, then there will be no recognizable patterns from which to construct evolutionary trees).

- Carl Woese chose the small-subunit rRNA as the gene used to infer the universal phylogeny. This gene was chosen because its structure is very similar in all organisms and its function is the same in all organisms. If your small-subunit rRNA does not function properly, you will not survive. Thus this gene is highly conserved and has been found useful in building the universal phylogeny.

- Figure 14.16 on page 491 shows a dendogram of the universal phylogeny based on the small-subunit rRNA. What is immediately noteworthy about this phylogeny is that the archaebacteria (called Archaea in the figure, the difference in nomenclature will be explained shortly) are more closely related to eukaryotes than eubacteria i.e., true bacteria (called Bacteria in the diagram, again differences in nomenclature are explained below). Archaebacteria live in extreme environments (temperatures close to boiling, extreme salinities and pressure). These types of environments are thought to have been prevalent on the early earth. It was commonly thought that the cenancestor would have been similar to the archaebacteria. In fact the name archae is from the Greek word for ancient and this fact underscores that scientists thought that the archaebacteria was “ancient bacteria.” It was a surprise to find that the archaebacteria are actually more closely related to the eukaryotes than they are to the eubacteria. Because of this fact, scientists renamed the archaebacteria, Archaea, in order to highlight the fact that these organisms are bacteria like only superficially, not phylogenetically. In this phylogeny, three “domains,” the Bacteria, the Archaea and the Eukarya, replace the five kingdom approach of classic taxonomy. This is a fundamental shift in our thinking of how life evolved.

- So what conclusions about the cenancestor can be drawn, if any? Using the principle of parsimony (the simplest explanation is usually the correct one), we can make some deductions. First, the cenancestor probably used DNA. This can be concluded from the fact that the Bacteria, Archaea, and Eukaryotes all use DNA. The alternative is that all three groups independently evolved the use of DNA. The fact that DNA-dependent RNA polymerases, and DNA polymerases are similar in all three domains (i.e., Bacteria, Archaea and Eukaryotes) suggest that the use of DNA is due to a common ancestor, and not convergent evolution. A picture of the
cenancestor begins to form of a highly evolved organism that already stored its genetic material in DNA and transcribed and translated it into proteins much as modern organisms do.

- But what did it look like? Here I will give my opinion. The early earth would have been a relatively hostile place to live. The early earth was geologically very active (much more so than today’s earth) and bombarded by meteors. It seems logical to assume that the cenancestor lived in this harsh environment. Thus the cenancestor may have been more similar to the modern Archaea. As more stable environments formed, some populations spread out and colonized these environments forming modern bacteria. The Archaea evolved in the dwindling pockets where early earth conditions still prevailed, e.g., hot springs. But modern Bacteria and Archaea are probably only refined versions of the cenancestor.

- Eukaryotes on the other hand are much different from Bacteria and Archaea. Eukaryotes have organelles, their genomes are in the form of linear chromosomes housed in the nucleus, not singular circular chromosomes without a nucleus as in Bacteria and Archaea. The genomes of eukaryotes have introns and exons (intervening and expressed sequences) in there genomes, Bacteria and Archaea do not. Many eukaryotes are multicellular with differentiated cells and tissues.

- So where did the eukaryotes come from? The answer, surprisingly, is that Eukaryotes evolved from the Archaea, and the Bacteria. Lynn Margulis first proposed the endosymbiotic theory for the evolution of eukaryotes. What she suggested is that organelles in modern eukaryotic cells were once prokaryotic cells that lived symbiotically inside other cells. These cells became so dependent upon their hosts that they lost all ability to survive outside of their hosts. Her evidence was that organelles such as mitochondria and chloroplasts “look” like bacteria. They also have their own genome, which consists of a single, circular piece of DNA, just like bacteria.

- To test this hypothesis scientists sequenced the small-subunit rRNA gene within mitochondrial and chloroplast genomes. If organelles evolved independently from within the eukaryotes, then the organelle’s genes should fall out within the eukaryotes. If organelles evolved as the result of symbiosis, then their genes should fall out within the bacteria. The answer can be seen on figure 14.24. Mitochondria are closely related to the proteobacteria (purple bacteria). Chloroplasts are closely related to cyanobacteria (blue-green algae). In an evolutionary sense, mitochondria are proteobacteria and chloroplasts are cyanobacteria.
• The small subunit rRNA research also suggests that the host of these endosymbionts was an Archaea. Thus, eukaryotes and ultimately even us, evolved from a symbiotic relationship between an extreme loving group of organisms and bacteria.