Origin of Life

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GLOSSARY

***α*-helix** A polypeptide structure in which amino acids spiral around a central axis. There are 3.6 amino acids per turn.

***β*-strand** A polypeptide structure in which the backbone zigzags and the side groups of the individual amino acids stick out on opposite sides.

exon The portion of an RNA molecule that is retained in final product and usually codes for a protein.

intron The portion of an RNA molecule that is cut out so that exons can be ligated together.

LIFE arose on Earth about 4 billion years ago and has flourished ever since. Over a hundred years ago, Charles Darwin pointed out that natural selection can lead to the origin of species, but he was unable to conceive of processes that could lead to the original appearance of life. More recently, A. I. Oparin, J. B. S. Haldane, S. Miller, M. Eigen and others have considered sets of chemical reactions that might lead to self-perpetuating systems. Their suggestions are based more on plausibility than on any new data from the dawn of life, but stay rigorously within the bounds set by the laws of physical chemistry. The surface of our planet is now teeming with life, but when it first condensed, about 4 billion years ago, there were only rocks, sand, and water in an atmosphere rich in nitrogen, methane, ammonia, and carbon dioxide, and devoid of oxygen. For a few hundred million years, Earth was bombarded by meteorites as it swept up the rocks in its orbit around the sun. Then the surface cooled down and life evolved. There are fossil traces of bacteria in rocks that are 3.5 billion years old. The conditions on Earth seem to have been favorable for the rapid evolution of life. We do not know exactly how the first microorganisms formed on the young planet long ago, but we know what it takes for an organism to live under conditions that prevailed billions of years ago. The early steps took place so long ago that there is little hope that they left any traces. However, the chemical processes that may have generated the building blocks for life can be carried out in test tubes to determine which ones are plausible and which ones are not. Biologists are beginning to understand some of the steps that may have led to life as we know it. We can link these together into a series that leads to the origin of life.

I. THE NATURE OF LIFE

We all agree that rocks are not alive. They do not grow, divide, make more rocks, or evolve into different-shaped rocks. Some semicrystalline clays of aluminum silicate can dissolve in water and reform when the water evaporates. The form of the original material determines the form of subsequent deposits to some extent, but the microscopic structures are ephemeral and subject to many outside forces. Clays cannot control their forms sufficiently to be considered alive. Living things must have the ability to grow and reproduce themselves accurately in the

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proper environment. All living organisms are constructed of cells in which the components necessary for survival and reproduction are surrounded by a protective layer of lipids and proteins. The information necessary for making all the vital components and arranging them in their proper places is carried inside in the form of long polymers of nucleic acids.

A. Cells

Cells come in many sizes and shapes, from bacteria that are less than 1 μm long to meter-wide fungal cells with thousands of nuclei. Most cells are no bigger than 10 μm across. Mammals and trees are just aggregates of a huge number of tiny cells. The earliest cells in the fossil record appear to be filamentous bacteria about 5 μm long. Larger cells and organisms do not appear in the fossil record until several billion years later. It seems that for most of the history of life of Earth, bacteria and other microorganisms have been the whole story. When considering the origin of life, we have the goal of seeing how the first bacterium might have arisen. The subsequent evolution of large organisms depended on the bacteria that preceded them.

Bacteria are defined as cells without internal compartments. The first cells that we would consider alive probably had a single membrane made of lipids and some proteins surrounding them. Later, many of these cells gave rise to bacteria with strong cell walls surrounding their outer membrane, but this structure does not seem to be absolutely essential because some bacteria survive today without a cell wall. Putting all the components in a membrane-enclosed space keeps them together and lets them evolve as an integrated system.

B. Powering the Process

The cellular membrane is also used to convert available energy sources into the chemical form that is most commonly used by all living cells, ATP. When a cell finds itself in an environment with more protons outside than inside, it can let a few protons in at a time by association with a protein embedded in the membrane. If this protein is coupled to an enzyme on the inside of the cell that has the ability to convert ADP to ATP, then the cell can generate ATP in this way. In fact, the coupling of proton-motive force to phosphorylation is the major source of biological energy in every organism today. It is the powerhouse of photosynthesis.

A cell cannot rely on encountering an environment with proton potential whenever it needs it, and so most cells have evolved a mechanism that lowers the internal concentration of protons by pumping them out. They will then have a lower concentration of protons inside than outside. However, pumping against a gradient takes energy and something has to be coupled to the proton pump for it to work. Cells have evolved a wide variety of ways to drive proton pumps, but the most prevalent uses the energy of sunlight. Pigments inside cells absorb light energy and use it to drive unfavorable reactions. Pigments such as chlorophyll are embedded in membranes and are coupled to proteins that drive protons against the concentration gradient. The cell can then let some protons back in through the proteins that are coupled to ADP phosphorylation and thereby generate ATP.

ATP is a very unstable molecule that can easily be activated to donate a phosphate to another molecule. Many proteins function as enzymes that catalyze such reactions. The catalytic site of an enzyme of this type is made from six repeats of about 40 amino acids that are strung together in a particular sequence such that they spontaneously take up the specific shape that has been called the mononucleotide binding fold. One-half of the chain of amino acids in the active site of the enzyme forms α-helices and the other half forms β-sheets. These secondary structures are the most stable states that the particular amino acids sequence of these enzymes can take, and so they always form spontaneously. Together the α-helices and the β-sheets make a pocket in which ATP is held in just the right orientation for it to react with another molecule held close by. The reaction then goes much faster than in the absence of the enzyme.

Charged molecules such as sugar phosphates cannot cross the lipid membrane. This makes it hard for a cell to take them up from the environment, but it is useful for concentrating them inside a cell once they are made in an enzyme-catalyzed reaction. Sugar

phosphates can be used later as a source of energy. Cells can take up uncharged molecules such as glucose from the environment or synthesize them from simpler molecules that may be available. To keep glucose in the cell, it is often necessary to add a phosphate from ATP and thereby convert it to glucose-phosphate, which will stay in the cell. When more ATP is needed, sugar phosphates and other such molecules can be metabolized in reactions catalyzed by specific enzymes into other molecules that will be able to donate the phosphate to ADP and make new ATP. These reactions go on, back and forth, in every living thing all the time.

C. Growing

Growth is one of the attributes we look for when we are trying to decide if something is alive. Even before there were living cells, biological molecules were growing and dividing. Proteins and nucleic acids make up most of the living material of cells and these have been synthesized since the earliest times. Proteins are long chains of amino acids of defined sequences. The order of amino acids, which can have any one of the 20 distinct amino acids at each position, is rigorously prescribed as the protein is synthesized. This process is carried out on the surface of ribosomes under the direction of specific messenger RNAs (mRNAs); each mRNA molecule directs the synthesis of only one of the thousands of distinct proteins found in living cells. The messenger RNA is itself a long chain of nucleic acid bases in which the order of the four bases, adenine (A), guanine (G), uracil (U), or cytidine (C), is also prescribed. When a new protein is synthesized, the code carried in the sequence of bases of the mRNA is translated by transfer RNA (tRNA) molecules that bind to three bases in the mRNA at a time. There are at least 20 tRNAs, each one specific for a certain sequence of bases in the mRNA (such as GGC) and each one carrying a specific amino acid that can be added to the growing peptide. In this way, a mRNA molecule that is 300 bases long directs the synthesis of a new molecule of a protein that is 100 amino acids long. Cells grow by accumulating proteins and other macromolecules.

The synthesis of the nucleic acids, RNA and DNA, is simpler than protein synthesis. The sequence of bases in the new polymer is directly determined in a one-to-one fashion by the sequence in another polymer as a result of the complementarity of the bases, themselves. For instance, whenever there is a G at a specific position in the template molecule, a C is added at that position to the growing molecule; likewise, C specifies G, A specifies U, and U specifies A. The chemistry of the bases is such that G-C and A-U pairs are favored over all others. Therefore, nucleic acids can direct the synthesis of complementary copies without any participation of proteins, although enzymes usually participate to speed up the reaction. The complementary copy can then direct the synthesis of copies of the original sequence.

D. Reproducing

When a cell has doubled in size, it can divide in two to generate copies of its former self. Cell division is a complex process in bacteria and other microorganisms that carefully positions equal amounts of all the essential components in each daughter cell. However, it should be kept in mind that bacteria have been selected over billions of years to reproduce at optimal rates, as well as to survive the competition of other microorganisms. When life was first evolving, competition was less fierce and any reproduction was a major success. The chief concern was to retain enough of the interacting molecules that sometimes both daughters were able to continue growing.

A cell with 100 RNA molecules coding for a certain enzyme can divide roughly in half and generate two cells each with about 50 molecules of the RNA. Sometimes one might get 70 and the other 30 molecules, but because RNA can replicate and direct the synthesis of new copies, any imbalance can be corrected. Modern-day replication is complex because there are thousands of highly selected genes in even the simplest organism and it is necessary to link a single copy of each into one long molecule of DNA. This chromosome is replicated from one end to the other before a copy is given to each daughter cell. DNA-based reproduction appears to have evolved to replace RNA-based reproduction at some time early in the history of life. The initial genes were probably exclusively RNA molecules, and reproduction was not quite as accurate as it is now.

Random errors generate the variants from which improvements are selected. Evolution depends on mutations that occur during reproduction, but success depends on accurately passing on the improvements that might have occurred. A balance has to be struck in which the rate of mutation is high enough to generate new stratagies for survival and yet low enough that the lucky few can become the lucky many. The replication of DNA in modern bacteria is highly accurate, making only one mistake every 10^8 bases. This level of accuracy is achieved by careful proofreading enzymes that correct most errors. Such meticulousness is essential in highly adapted organisms, but was probably unnecessary in the simpler cells that first arose. A mistake every 10^4 bases could have been easily tolerated.

II. THE PREBIOTIC SOUP

A. The Spark Flask

In 1953, when he was a graduate student, Stanley Miller carried out a simple experiment at the suggestion of his adviser, Harold Urey. For several days he let a spark discharge every few seconds in an atmosphere of methane, ammonia, hydrogen, carbon monoxide, and water. This is a reducing environment like the one that is thought to have covered this planet during the first few billion years. At the end of the experiment, Miller analyzed the contents of the flask. The warm solution contained high concentrations of more than 10 distinct amino acids, as well as many other interesting compounds that could form biologically important molecules. The concentration of many of the compounds was greater than 1 μM. Under slightly different conditions, the purine and pyrimidine bases that go into making nucleic acids were made from equally simple starting materials. Likewise, sugars and lipids will form in reactions catalyzed by metalic ions. These results make it clear that the prebiotic conditions favored the formation of the subunits of proteins and nucleic acids. At times, these ingredients could be concentrated when puddles or droplets dried up or when the surrounding water froze. In some locales the subunits coated the surface of clays and minerals, where they reacted with each other to form even more complex molecules. One of the molecules with the most potential may have been the precursor to RNA. The nucleic acid bases could condense with glycerol phosphate and then be strung together in random order. Such acyclic nucleic acids have been made in the laboratory and shown to direct the synthesis of complementary copies.

B. The RNA World

At some point before the appearance of life, nucleic acid bases linked to the cyclic sugar D-ribose were polymerized into short chains. The sequence of bases in these oligomers may have been partially determined by their hybridization to complementary sequences in preexisting acyclic nucleic acids and thereby derived some hereditary information from the sequences that preceded them. There are viruses alive today in which the genes are carried in the form of RNA rather than DNA.

Individual nucleic acid bases attached to riboses can spontaneously add to the end of an RNA chain, thereby elongating it. The choice of which of the four bases is attached at any given point is determined by the complementarity rules (G pairs with C; A pairs with U). Therefore, when a short RNA molecule is bound to a slightly longer one, the shorter one will grow by adding the complementary base.

This reaction is driven by the energy inherent in the complementation of bases with each other.

So far we have not invoked any catalysts at all. However, it turns out that RNA itself has the ability to catalyze the making and breaking of the bonds between monomers and polymers by acting as an intermediate. Such RNA molecules have been called ribozymes and several have been found to still be functioning in present-day cells. Ribozymes have been shown to lead to the polymerization of about 30 bases per hour. The polymers that are made are all linked correctly and have the expected sequences 99% of the time. Thus, RNA can make RNA.

RNA can also catalyze the transfer of phosphates between unrelated molecules and so could have been the central player in a set of simple but interactive

reactions that occurred before peptides joined the story. This RNA world would have had considerable stability as well as a limited ability to evolve greater complexity. However, when RNA functions together with single amino acids or short peptides, the level of complexity can go up rapidly and greater specificity can be given to the system as a whole.

C. Coding for Peptides

Peptides cannot replicate themselves and so must depend on sequences in RNA to determine their amino acid sequences. Translating base sequences in RNA into amino acid sequences in peptides is facilitated by tRNAs. These short polymers take up unique three-dimensional structures that distinguish them so that only a single type of amino acid is added to a specific tRNA. A given tRNA molecule then binds to three adjacent bases in the coding RNA using the complementarity rules and presents its amino acid for addition to a growing peptide. When the system was just getting started, the function of tRNAs might have been played by RNA polymers less than 15 bases long in which the sequence was self-complementary. That is, they could bend back on themselves and form the base pairs that stabilize their three-dimensional structure. The end of the hairpin could accept amino acids, whereas the bases in the loop at the top could bind to the template RNA molecule as they decoded it.

One of the biggest problems in understanding how translation could have occurred before there was life on Earth is to account for the specific attachment of only one kind of amino acid on each kind of tRNA. The structure of tRNA itself is not sufficient for this specificity and so it is likely that randomly synthesized peptides were involved from the start and that by chance some of the systems coded for these very peptides. When this occurred, the first positive feedback system was established and the system could rapidly expand.

When amino acids dry out on rocks and clays, they will sometimes polymerize into random polymers. There may have been other environments as well in which short polymers of 10 to 12 amino acids were formed. Some of these peptides probably had the ability to bind certain amino acids, as well as a specific hairpin RNA that could serve as a tRNA. Those that increased the rate of aminoacyl tRNA formation stood a chance of entering an autocatalytic cycle (Fig. 1). However, they had to be in the same microenvironment with an RNA that happened to have a sequence coding for a similar peptide. Because millions of sequences were being made and broken down all the time, it probably did not take long for this chance to come along. The sequence did not have to code for exactly the same sequence of amino acids in the peptide because many of the 20 amino acids have very similar chemical properties. For instance, glycine, valine, and alanine are all uncharged hydrophobic amino acids whereas both asparatate and glutamate are negatively charged hydrophilic amino acids. As long as one of the hydrophobic amino acids was encoded, it probably did not matter much which one it was. Similarly, for the hydrophilic amino acids, the system only had to distinguish from among about five kinds of amino acids, and if it was correct only 10% of the time, it could still expand. Before the appearance of life, there were no enzymes that would break down peptides or nucleic acids, and so even very inefficient, primitive systems had the time to carry out each step. The biggest problem was keeping the system stable. Error catastophies in which one weak link leads to the next undoubtedly destroyed many promising early systems.

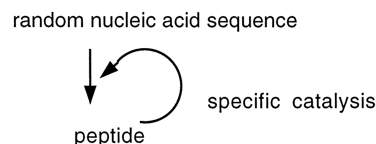

Fig. 1. An autocatalytic cycle. If a peptide happens to favor its own synthesis, more and more of the peptide will be made. Randomly generated nucleic acid sequences partially specified certain peptides that entered into such cycles.

D. Hypercycles

The solution to finding greater stability came from linking cycles together into hypercycles. If some peptides increased the selectivity of the addition of amino acids to tRNAs, whereas others increased the rate of replication of RNAs and peptides, they would all help to make more of the others. The increased

fidelity of translation would help make the peptides that made more peptides and RNAs, as well as the peptides responsible for the increased selectivity. Similarly, the peptide that made more RNA would help make more copies of the RNA encoding itself, as well as the tRNAs and the RNAs that encoded the peptides for adding the proper type of amino acid (Fig. 2). This kind of bootstrapping could lead to relatively rapid increases in both stability and specificity.

The original hairpin RNA molecules of a dozen bases grew longer and were able to take up more specific shapes. Modern tRNAs are about 60 nucleotides long and have specific sequences that result in cloverleaf shapes because the bases along the stems are complementary. Each tRNA is charged by a specific amino acid and uses three bases in its anticodon to bind to three complementary bases in mRNA. Hairpin RNAs in early hypercycles may have been specific to only one of the bases in a triplet codon, but still provided some sequence specificity to the newly made peptides. There has always been a reward for greater accuracy, and when the tRNAs of one hypercycle happened to use two or even three bases for translational specificity, they all benefitted. The present-day code by which the sequence of bases in RNA is translated into proteins gives some clues as to how it happened to arise. The first base in the codons for glycine, valine, alanine, aspartate, and glutamate is G in all cases. It certainly looks as if this system evolved from one in which any tRNA that recognized that G would add one of these amino

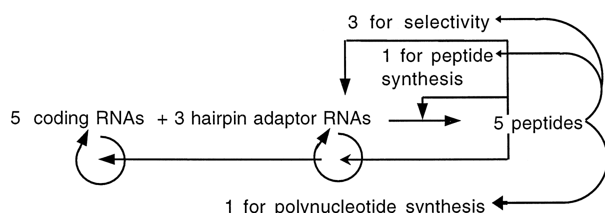

Fig. 2. An integrated set of autocatalytic cycles. Eight randomly generated RNAs could be integrated into a hypercycle by the function of the peptides they happened to encode. If the products of three RNAs increased the accuracy of translation of the codons used by all the five coding RNAs and the product of another increased the replication of them all, then the system would benefit from using three hairpin RNAs as tRNAs.

acids as long as the second base in the codon was not A. Codons with the sequence GAN (where N is any of the four bases) would be recognized by tRNAs charged with either aspartate or glutamate. Later on, tRNAs evolved with specificity to the other bases in the second position and allowed glycine, valine, and alanine to be distinguished. At a later time, when the system was already greatly improved, tRNAs could evolve with specificity to the third base in the codons such that aspartate and glutamate could be distinguished. Such stepwise increases in the specificity of coding could have gradually brought each of the 64 codons into use. The complete genetic code did not have to arise all at once.

The same is true of the activating enzymes that add specific amino acids to tRNAs. They could have gradually improved on what was initially a very inaccurate system. The examination of modern-day activating enzymes shows that they evolved from more primitive ones that worked on several different amino acids. There may have been only a few of these primitive catalysts that were then duplicated and diverged to gave rise to the highly specific enzymes we have today.

When hypercycles started to have more than a few dozen interacting components, stability was more limited by the environment than by the system itself. Mixing could disperse the needed components or bring in ones less adapted to the particular system. Even before this crisis was reached, lipid droplets enclosed small volumes. Some of these contained all of the interacting components of a successful hypercycle and went on to develop new properties.

III. COUPLED SYSTEMS

A. Primitive Metabolism

Droplets that encompassed semistable hypercycles generating peptides and nucleic acids could grow and evolve if they were able to manipulate their environment. Organic reactions that had been catalyzed by simple metal ions or prebiotically generated co-enzymes such as nicotinamide (vitamin B3) or pyridoxal phosphate (vitamin B6) could be improved by peptides that bound these cofactors. RNA mole-

cules that happened to code for such peptides might have been trapped by the droplets or generated by the error-prone replication system. They did not have to be long sequences; 25 to 30 bases would do because the peptide asparagine, phenylalanine, asparagine, proline, histidine, lysine, tryptophan (or NFNPHKW in the standard one-letter abbrevations) is sufficient to bind pyridoxal phosphate and is actually found in such enzymes of both vertebrates and invertebrates. It is likely that similar peptides linked to only a few other amino acids would bring pyridoxal phosphate together with substrates for a variety of reactions. Other short peptides might bind zinc or iron atoms and catalyze certain reactions. The most useful would be those that provided the substrates for protein and nucleic acid synthesis. Equally important would be peptides that could derive energy from available sources.

A peptide that bound magnesium ions and ADP could convert ADP to ATP by coupling the reaction to the dephosphorylation of diphosphoglycerate. The product, phosphoglycerate, could be further metabolized by distinct but related peptides to generate another molecule of ATP and pyruvate. All that it takes to bind ADP is a mononucleotide-binding fold consisting of two dozen amino acids in a sequence that will spontaneously form a β-strand connected to an α-helix followed by another β-strand. If the connecting sequence has three glycines and a properly positioned lysine, a hydrophobic pocket will be made. The aggregation of six such molecules would form a barrel into which ADP would fit tightly. Today, these six peptides are all linked in a longer protein, but originally they may have associated only by the interaction of their hydrophobic surfaces.

Coupling the oxidation or reduction of a wide variety of compounds to the phosphorylation of ADP would generate ATP, which could be used in biosynthetic reactions even before the energy of sunlight was harnessed. However, fairly early on a droplet that could derive energy directly from trapping the energy in sunlight had a big advantage because it would be more independent of the environment. The first photophosphorylations may have coupled an ADP-binding protein to a heme-binding protein that was activated by sunlight. Whether these coupled peptides had to be embedded in the lipid layer that surrounded the droplet is not clear, but that might not be improbable in any case. With a steady source of energy, peptide and nucleic acid synthesis could proceed rapidly. Competition between droplets for raw materials then became the decisive factor.

B. Protocells

Simple lipid droplets containing a few peptides that increased the rates of a few reactions cannot be considered alive. The early peptides were nowhere near as effective catalysts as are modern enzymes and did not have the exquisite specificity we have come to appreciate. Moreover, the replication system was so error prone that no two peptides were exactly alike. Nevertheless, such droplets could increase in mass, replicate some of the essential components, and divide them among smaller droplets when they broke up. Each droplet then became a partially independent unit that could respond to selective pressures. The more stable, more accurate systems lasted longer and multiplied. Controlling the properties of the droplets themselves may have been the next major step toward the origin of life.

There would be strong selection for membrane proteins that could affect the physical properties of the droplets. Hydrophobic peptides are spontaneously embedded in lipid bilayers and change the characteristics of the membrane. For instance, some membrane proteins facilitate the transport of ions, such as potassium or protons, whereas others can pump glucose or amino acids into cells. Peptides with similar properties may have been early arrivals on the scene. A droplet able to scavenge the environment had a strong advantage. Of course, if it were to pass on this ability to subsequent droplets, it would have to have an RNA encoding the peptide that gave it this advantage.

Membrane proteins could also serve as anvils on which to hammer out new peptides. Template RNA molecules could be held to the inner surface of the droplet while tRNAs bound to them and donated their amino acids to growing peptides. This function is now carried out on ribosomes within cells, but ribosomes are large complex organelles containing several RNA molecules and over 50 complex proteins. There had to be something simpler before there

TABLE I
Fifty Vital Sequences

1	Nucleic acid polymerase
2	Purine synthetases
2	Pyrimidine synthetases
1	Phosphotransferase
2	Peptide elongation factors
3	Ribosomal proteins
2	rRNAs
10	Amino actyl tRNA synthetases
10	tRNAs
4	Amino acid metabolic enzymes
2	Oxidation–reduction enzymes
5	Fermentation catalysts
4	Membrane proteins
2	Lipid metabolic enzymes

were ribosomes. The inner face of the droplets might have served this function when specialized peptides were embedded in the lipid layer.

As each advance was made, the system became more stable, more resilient, and better able to multiply than less-favored systems. A minimum of 50 vital sequences seems to have been necessary for a droplet to have had much of a hope of surviving (Table I). It would have been a complicated system, but still far from alive.

C. Survival by Replication

A droplet with 50 vital sequences would have to have hundreds of copies of each one to ensure that it would give at least one copy of each sequence to smaller droplets when it broke up. Further expansion of the genetic repertoire required a more orderly manner of distributing the information. All living cells use DNA rather than RNA for their genetic material. The genes are strung together in a few long molecules that can be easily distributed to daughter cells. Moreover, DNA is almost always double-stranded, so the information is carried redundantly in the two complementary strands. If one strand is harmed, it can be repaired with no loss of information. There are several other advantages of DNA over RNA, and the early protocells soon shifted over to using it. Besides using 2′ deoxyribose-linked nucleic acid bases, DNA uses thymine rather than uridine. The enzyme that converts uridylate to thymidylate evolved long before bacteria started to diverge and has been conserved ever since. There are only eight differences in the active site found in the bacteria *Lactobacillus casei* and *Escherichia coli,* and not many more in the active site found in humans. The active site is about 30 amino acids long and uses a hydrogen bond between cysteine and arginine to catalyze the reaction. A similar short peptide probably carried out this same reaction before the dawn of cells.

Once the sequences of RNA were copied into long molecules of DNA, both the number and the length of genes could expand. Each gene could be several hundred bases long and code for proteins of 100 or so amino acids that had greater stability and specificity. The rate of growth and division could increase as several hundred genes were strung together and distributed equally to daughter cells.

When more than half of the progeny cells were as functional as the original cells, the population would expand. At this point, the seas rapidly became filled with cells all competing with each other. Those that were able to replicate faster than others had a distinct advantage. When weaker cells broke, other cells scavenged parts from the wreckage. It is hard to conceive of the huge number of cells that can live on this planet. There are over 10^{20} ml in just the top 10 m of the oceans and there can be millions of cells in each milliliter. With 10^{26} cells growing and dividing every day, events as rare as 1 in 10^{30} would occur every century. When a rare cell acquired a strong advantage, its progeny could take over the world in a few hundred years, and all subsequent improvements would take place in the background of the new improved model. Without much question, these cells were alive.

IV. THE EARLIEST CELLS

A. Common Descent

It is impossible to be certain of the steps that led to the first cells, but it is possible to know something about what the first cells were like because all living things are descended from then. There is ample evi-

dence that one line came to predominate and gave rise to all subsequent organisms. All cells use the same triplet code for the translation of nucleic acid sequences into protein sequences as well as the same punctuation marks, and all use the same basic metabolic processes. Even the sequences of amino acids in the active sites of some enzymes have been conserved since earliest times. For instance, the following sequences are found in triose phosphate isomerase, the enzyme that makes 3-phosphoglycerate:

Escherichia coli	QGAAAFEGAV	IAYEPVWAIG	TGKSATPAQ
Bacillus stearothermophilus	QLTPQEVKII	LAYEPLWAIG	TGKSSTPAQ
Rabbit	DNVKDWSKVV	LAYEPVWAIG	TGKTATPQQ
Fish	DDVKDWSKVV	LAYEPVWAIG	TGKTASPQQ
Corn	EKIKDWSNVV	VATEPVWAIG	TGKVATPAQ

Although there have been changes in the amino acid sequence of this enzyme over the last few billion years, a run of 12 amino acids (AYEPVWAIGTGK), as well as other components of the active site, have remained practically the same. The chance occurrence of 12 amino acids in a row being identical is 20^{12}, because there are 20 different amino acids that could be at any one of the positions. Together with the other similarities in the primary sequence of triose phosphate isomerase in these bacteria, vertebrates, and plant, there is no question that they are descended from a common sequence that was functioning even before bacteria had evolved. The only other possible route to such sequences would be convergence from independently evolved sequences. Convergence of more than a dozen amino acids would be so rare that it can be safely ignored. Selective forces appear to have kept this and other portions of triose phosphate isomerase almost unchanged for billions of generations. Humans that have a single amino acid change in the active site of triose phosphate isomerase suffer from hemolytic anemia and neuromuscular disorders. Their chance of passing on the varient triose phosphate isomerase is exceedingly low.

There are many other enzymes in which the active site has remained almost the same since it arose in the earliest cells. These include pyruvate kinase, thymidylate synthetase, several transcarbamylases and dehydrogenases, the CO_2-fixing enzyme ribulose-bisphosphate carboxylase, the nucleic acid polymerases, and proteins involved in electron transfer such as the ferridoxins. We can be fairly sure that genes encoding very similar proteins were functioning in the earliest cells. Copies of these genes have duplicated in the genomes of various organisms and given rise to distinct but related proteins. Thus, the whole genetic repertoire of bacteria and larger organisms is derived from the first few hundred genes of the cell line that first populated Earth.

Most improvements in early genes came from adding on pieces of other genes, rather than from changing the core sequence. This process of accretion can be seen in the mosaic structure of many ancient enzymes. They often have a common sequence flanked by sequences found in unrelated enzymes as well as pieces that have diverged so much that their origins can no longer be recognized. This mix-and-match approach is the consequence of rare errors in replication of DNA that result in fusion of one sequence to another. Now and then, such a translocation resulted in an advantage to the cell and the mutation was fixed in the population. The eminant microbiologist Francois Jacob has described evolution working as a tinkerer. Natural selection can only work with the random mutations that are present and so it is impossible to aim for a set goal. Mutations that seriously change a vital sequence are lethal, but those that just fiddle with what is working and happen to make it work better can be saved for use by posterity.

B. Introns

The fusion of sequences to generate complex genes is a powerful mechanism that can lead to improved products without compromising existing advantages. But it has serious side effect. The fusions seldom start or end exactly where coding regions do, and so there will be intervening sequences separating the two portions. If there are signals for the termination of translation in the intervening sequence, the mRNA will not be fully translated. However, certain RNA molecules have the ability to splice out portions of their own sequences and fuse two or more coding portions or exons. Fused genes that happen to make a self-splicing RNA that removes introns can function as well as the far rarer fusion genes, in which the

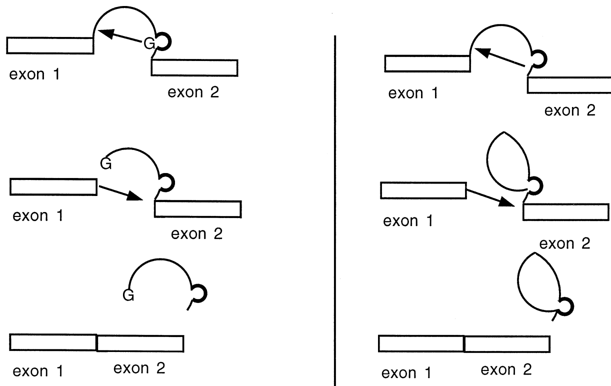

Fig. 3. Self-splicing RNAs. There are two related mechanisms by which RNAs catalyze the removal of their own introns. In Group I RNAs (left), a free guanine (G) is bound and used to cleave the 5′-end. In Group II RNAs (right), an internal adenosine attacks the exon–intron junction and then forms a lariat of the intron. The cleavage of the 3′-junction and ligation of the exons forms the final functional product. Most mRNAs belong to Group II.

joint was perfectly in phase. Although introns are prevalent in eukaryotic organisms, they are rare in bacteria. Until recently, this was taken as evidence that exon splicing only evolved at the time of appearance of eukaryotic cells about 1 billion years ago. However, during the late 1990s, a number of bacterial genes have been found to carry introns that are removed by self-splicing (Fig. 3). It is becoming clear that many genes may have initially carried introns, but that there was a strong selective pressure within the last billion years for the replacement of these genes by copies derived from their processed (spliced) mRNAs. A few remain in anaerobic bacteria and in the viruses of aerobic bacteria.

C. Regulation

A system without controls has little chance of survival. The integration of coupled processes requires that the individual components be able to regulate each other to arrive at the most selectively advantageous mix. Early cells undoubtedly had mechanisms for the regulation of both the transcription of mRNA and translation of proteins. We can see the kinds of proteins that may have mediated these controls by comparing transcriptional and translational regulators found in both bacteria and eukaryotic cells.

One of the motifs found in DNA binding proteins is called a zinc finger. These proteins have a section about 20 amino acids long in which four cysteines or histidines are so positioned that they can all bond to a central zinc ion. This forms a loop or "finger" that binds to three bases in the major groove of DNA and holds the protein near those genes that have this particular sequence of three bases. There are usually several zinc fingers in each DNA binding protein, so that 12–15 bases are used to provide specificity. A sequence of 15 bases will only occur randomly once in a billion bases ($4^{15} \approx 10^9$). This combinatorial use of short sequences is found in almost every case in which a protein associates with a specific region of DNA.

Another motif found in DNA binding proteins is the helix-turn-helix. In these proteins, a sequence of a dozen amino acids forms an α-helix that can fit in the major groove of DNA and associate with four bases. The second helix is positioned at right angles to the first one and holds it in place. These proteins usually function as dimers recogizing palindromic sequences of eight bases. When such a protein is bound to DNA, it can either facilitate or inhibit transcription from nearby genes, depending on the the nature of the rest of the DNA-binding protein. Because a given eight-base sequence occurs every 10,000 bases or so along a DNA chain, there are thousands of binding sites in any genome. The binding of a helix-turn-helix protein to one of these is stabilized by interactions with other DNA-binding proteins that have recognition sites nearby. Transcriptional initiation is dependent on the formation of a complex consisting of several independent proteins, all of which have to have binding sites within several hundred bases of the gene in question.

Proteins that bind RNA are less well understood and common structural motifs have not been recognized. They appear to recognize specific RNAs on the basis of secondary structures that the RNA take up by internal complementarity. If the secondary structure is disrupted by interactions with other RNA-binding proteins or with ribosomes during translation, then the recognition site is removed. Thus, the regulation of translation also works in a

combinatorial fashion that can pick out one mRNA sequence from the thousands of different RNA sequences in a cell.

Feedback loops form when a macromolecular or metabolic process interacts with a specific regulatory mechanism controlling another gene. The link is usually made through a small molecule effector such as cAMP or cGMP. The activity of DNA-binding proteins is controlled by interactions with such effectors at independent sites in the regulatory protein. This is usually the first step in a hierarchical control mechanism. Regulation can come in at a higher level as well, by controlling the genes for the regulatory proteins themselves. In this way, the function of thousands of genes can be carefully orchestrated to result in a harmonious response to the state the cell finds itself in at any given time.

V. MICROBIAL DIVERSIFICATION

The complete genomes of a dozen microorganisms have been sequenced such that all their genes can now be inspected. The comparison of the most closely related genes in a variety of bacteria confirmed the ancient divergence of the eubacteria and archaebacteria about 3 billion years ago. For the first billion years of life, genes may have been passed among different bacteria so frequently that the concept of separate species would not apply. Species that arose from this pool of shared genes gradually put up barriers to lateral transfer and proceeded to evolve for the most part independently. As bacteria further specialized to exploit different environments, their genomes diverged more and more. About 2 billion years ago, it appears that endosymbiosis of an archaebacterium and a eubacterium gave rise to the eukaryotes and their organelles, respectively. Many genes were transfered to the nucleus, where they were organized into chromosomes.

The fossil record gives little to go on when all life was microbial, and so the timing of events that led to the deepest branching that separates the archaebacteria from the eubacteria and the more recent formation of the eukaryotes and major bacterial groups has had to be extrapolated from comparisons of the amino acid sequences in orthologous genes found in present-day members of these clades. Nevertheless, data are accumulating that gram-positive, gram-negative, and cyanobacteria diverged about the same time that an α-proteobacterium became established within an Archaea-like bacterium to give rise to eukaryotes. Some of the newly formed eukaryotes subsequently lost their mitochondria but retained many of the α-proteobacterial genes. As oxygen began to build up in the atmosphere, others diversified to give rise to protists, plants, fungi, and animals. Starting about 600 million years ago, the history of life on this planet can be traced in rocks where the variety of living things can be clearly seen in well-preserved fossils.

VI. CONCLUSION

Once an autocatalytic process was set in motion over 3.5 billion years ago, there was very little that could stop life from expanding, diversifying, and filling every conceivable niche on the surface of this planet. All life descends from the first cells that arose spontaneously from the prebiological soup. Those early cells were more like bacteria than they were like eukaryotes, but it is hard to say if they were more like archebacteria or eubacteria. There is no question that they were anaerobes because oxygen had not yet built up in the atmosphere. Photosynthetic bacteria released oxygen for several billion years before the capacity of Earth's surface to reduce it to iron oxides and other minerals was exhausted. Thereafter, aerobic bacteria and eukaryotes evolved from the existing anaerobes.

The wide distribution and impressive extent of stromatolites from the some of the oldest rocks on Earth attest to the ability of early bacteria to proliferate. Stromatolites are laminated rocks several meters in height that form when sand is trapped in the filaments of bacterial colonies. They have been found in formations over 3.5 billion years old in Australia, South Africa, the Sahara, Greenland, and Siberia. It is clear that life originated on this planet a few hundred million years after the oceans cooled and the meteoric

bombardment was over. For several billion years, life remained confined to single-cell organisms that resembled modern-day bacteria in many ways. During this long period, natural selection could work to fine-tune each gene for optimal adaption to an enormous number of specialized environments. Bacteria lived in seawater, freshwater, and brines. They lived in thermal hot springs at 100°C and in polar seas at 0°C.

When the atmosphere started to fill with oxygen, the bacteria that were not killed could capitalize on the chemical potential of the air and evolve aerobic metabolism. That allowed multicellular eukaryotic organisms such as humans to evolve. Most of our genetic heritage is derived from the bacteria of a billion years ago and theirs, in turn, was inherited from the earliest cells that had learned to survive with only a few hundred simple genes. When life first appeared, it was a fragile thing, but it had the power to evolve into almost anything.

See Also the Following Articles

Diversity, Microbial • DNA Replication • Evolution, Theory and Experiments • RNA Splicing, Bacterial

Bibliography

DeDuve, C. (1991). "Blueprint for a Cell." Patterson, Burlington, NC.

Feng, D-F., Cho, G., and Doolittle, R. F. (1997). Determining divergence times with a protein clock: Update and reevaluation. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13028–13033.

Huynen, M., and Bork, P. (1998). Measuring genome evolution. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5849–5856.

Joyce, G. (1998). Nucleic acid enzymes: Playing with a fuller deck. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5845–5847.

Loomis, W. F. (1988). "Four Billion Years." Sinauer, Sunderland, MA.

Miller, S., and Orgel, L. (1974). "Origins of Life of Earth." Prentice-Hall, Englewood Cliffs, NJ.

Rivera, M., Jain, R., Moore, J., and Lake, J. (1998). Genomic evidence for two functionally distinct gene classes. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6239–6244.