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PRE-BIOTIC EVOLUTION THE MISSING LINK OF BIOLOGICAL EVOLUTION

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PREFACE

Most of the scientific community has been gleefully watching as the scientific journals fill in areas of evolution *beyond* the first living cell, because we all are interested in how the magnificent biological world we live in came about. Like many other scientists, I have been content to do my plant-related research and to stand on the sidelines as our understanding of evolutional biology increased. My research as a plant molecular biologist over 5 decades demanded that I keep abreast of the developments at the molecular level. The plant journals can not cover the complete advances of molecular arrangements in other organisms and thus great clues can reside in the literature covering other eukaryotic organisms.

I found that journals such as Cell and then Molecular Cell were a good source of new concepts and the methodology of the associated research. Not only did the research in these journals answer questions, it further revealed the previously unknown complexity of biological processes. The kind of complexities that hit me hardest were processes such as blood coagulation, the role of polymerases in DNA replication and cell division, and the complex requirements of mutation linked DNA repair. I sat in wonder in the 50's as a geneticist from Sweden explained his model for how the DNA is replicated and separated in cell division. I wondered how all of the DNA strands a meter or so long and within a 10-micron space could be so perfectly separated within the 20 minutes of cell division. Who at that time would have suggested that the untangling involved cutting and reuniting the DNA strand at multiple places. I know that the term, intelligent design, is a bad term when used in connection with evolution, but how can anyone (particularly molecular biologists) not recognize that almost all aspect of a living cell involves an intelligent design. How we get from the elements of the periodic table to the first living cell is in my opinion the real "missing link" in understanding evolution. The "origin of life" topic, whether currently in relation to Mars, Saturn moons

or Earth is a serious topic for discussion and I am attempting to reiterate some of what is known about life as we research it. To those that may not know, biological life is not simply a matter of water, and a few elements coming together under harsh environmental conditions as NASA and some biologists would like you to visualize. I hope to demonstrate in this paper the complexity of what would have been required to assemble the first living cell. Such a simplistic view of the assembly of a living cell, revisited after 50 plus years of molecular biological research, can no longer account for the intricate development of what we currently know the cell's organization to be. The concept that life was created in this random way is currently facing much larger informational gaps than ever before. However, prebiotic evolution is still being presented to 1st year biology students as a solid concept.

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1. Introduction

There are some reasons why the evangelical explanation of creation is resisted by many within the scientific community. These reasons are obvious to most scientists. The biblical time line of creation and that obtained from carbon dating are in obvious conflict. The current crop of scientists understands the accuracy of carbon dating so they are inclined to defer from specifics of creation described biblically. The accuracy of the bible gathered from experiences and events, recorded nearly a century afterwards, would expectedly have inaccuracies. The bible authors did not possess the library and scientific tools that the current fields of science enjoy. Alternately, present day scientists with genome analyses have better insights of the complexities of biology than even those known in the era of Watson and Crick [1] or of Darwin [2]. I am dedicated to bring some of the complexities to the attention of those who have not had access to the more recent inner workings of biology.

2. Darwin's Observations and Hypothesis

The tools of Darwin allowed him to observe change in biological organisms that were so significant that taxonomists can readily see plausible developmental routes to new species in a process called "micro evolution" or "post-biotic evolution". Anthropologists have been reasonably successful in proposing routes of change in bone or feather structure, physiological capabilities, e.g. in the origin of birds [3], as one might visualize as occurring over the span of multimillion years resulting in the biological variation reported today. In fact, with single celled organisms even single mutational events can potentially lead to designated changes recognized by taxonomists as changes to a new species. The newly formed biological entities can potentially be improved in some way that may be beneficial to their existence, such as tolerance to physical or chemical environments, nicely bucking up Darwin's view on survival of the fittest. Anyone who has had antibiotics prescribed to them knows that bacteria have the potential to acquire resistance to most of the known antibiotics [4]. All of these changes require an intact living cell both before and after the mutational event. However, the real incompletely-understood-missing link is the processes that take elements of the periodic table elements to the first living cell [5].

	1 H 1.008																2 He 4.003
2	3 Li 6.94	4 Be 9.012											5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18
3	11 Na 22.99	12 Mg 24.31											13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.06	17 Cl 35.45	18 Ar 39.95
4	19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.88	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.39	31 Ga 69.72	32 Ge 72.64	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.79
5	37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.96	43 Tc (98)	44 Ru 101.1	45 Rh 102.9	46 Pd 106.4	47 Ag 107.9	48 Cd 112.4	49 In 114.8	50 Sn 118.7	51 Sb 121.8	52 Te 127.6	53 I 126.9	54 Xe 131.3
6	55 Cs 132.9	56 Ba 137.3	*	72 Hf 178.5	73 Ta 180.9	74 W 183.9	75 Re 186.2	76 Os 190.2	77 Ir 192.2	78 Pt 195.1	79 Au 197.0	80 Hg 200.5	81 Tl 204.38	82 Pb 207.2	83 Bi 209.0	84 Po (209)	85 At (210)	86 Rn (222)
7	87 Fr (223)	88 Ra (226)	**	104 Rf (267)	105 Db (268)	106 Sg (269)	107 Bh (270)	108 Hs (277)	109 Mt (278)	110 Ds (281)	111 Rg (282)	112 Cn (285)	113 Nh (286)	114 Fl (289)	115 Mc (289)	116 Lv (293)	117 Ts (294)	118 Og (294)

Periodic table or the elements printed with permission from the Los Alamos National Laboratory, Chemistry Division.

This first cell would have likely been a simple cell. For example, the oceanic bacterium *Pelagibacter ubique* has the *"smallest number of genes (1354 open reading frames) for any free-living organism. Has complete biosynthetic pathways for all 20 amino acids and all but a few cofactors. No pseudogenes, introns, transposons extrachromosomal elements, or Inteins, few paralogs and the shortest intergenic spacers yet observed for any specie".*

Starting from the periodic table of elements

Now where do we start from to get onto the ever changing developmental process? The "big boom" is for the physicists to sort out. How about starting with the elements of the periodic table. This small table of molecular reality covers all known atoms on earth and some known to exist in outer space, as well as some information on their reactive properties. What are the next steps?

Viewpoint of the 1950-60s

At this point I would like to confine my discussion to what I think were the most influential publications that suggested to the scientific community of 1950, and initially to me, that pre-biotic evolution started with the elements of the periodic chart. Connecting the dots together was considered in the 50s and 60s as a process that could be conceived based on some preliminary experimental data. Much of it is summarized in a special issue of *Scientific American* [6] that was an accumulation of previous *Scientific American* papers. The issue was

entitled “Life: origin and evolution”. W. H. Freemen and company, SanFrancisco. Included were articles with copyrights dated from 1952 through 1979.

Formation of organic molecules

There was a consensus at that time there was first a formation of organic molecules. There was little free oxygen but the development of simple organic compounds was thought possible in the absence of living organisms. This indeed can still happen, an example given is that volcanic eruptions bring metal carbides to the surface of the earth where they can combine with water vapor to yield simple compounds of carbon and hydrogen.

At that time S. L. Miller performed a simple experiment [7]. He mixed a combination of methane (CH_4) and ammonia (NH_3) and hydrogen in combination with an electrical spark and heat continuously for a week. The water that condensed in a side vessel contained some identifiable simple amino acids such as alanine and glycine and possibly aspartic acid and two others. This gave an initial indication for spontaneous formation of the organic compounds that are components of proteins.

In the C.E. Folsome-organized series of articles and essays [6] under the title, Life: Origin and evolution it related some of the thinking of the l950-1970s. “The origin of life” by Al Oparin, translated into English by George Wald in 1954 summarized some of the thinking on spontaneous generation. All of the conclusions involved the massive amount of time that must have been involved and was stated as follows: “ *Time is in fact the hero of the plot. The time with which we have to deal is of the order of two billion years. What we regard as impossible on the basis of human experience is meaningless here. Given so much time the impossible becomes possible, the possible probable, and the probable virtually certain. One only has to wait: time itself performs the miracles*”. Even though, scientists to become convinced, had to accept all of this on faith. As indicated above, Stanley Miller had unearthed a harsh-treatment scheme for obtaining a few amino acids that serve as building blocks of proteins in living cells. He stimulated thinking on questions such as, does the presence of traces of a few amino acids translate into potentially functioning proteins?

Protein function

The major biological function of protein is as an enzyme that can catalyze reactions. The living cells of the simplest, as well as the most complex organisms, have so many vital reactions that must be catalyzed such as those known to be involved with intermediary metabolism. This information can easily take up the total area of a 4x6 foot chart. Such charts used to be prominently presented in most biochemical laboratories (See photo, **Figure 1**). The construction of any one of these enzymes is certainly complex. Most of the 20 plus amino acids are present in the simplest enzyme and their sequence is what determines the protein’s secondary

structure and as well as the catalytic function. Often the protein molecule has a length of over 100 amino acids.

Self-assembly of proteins

Although the logics of self-assembly of life should have been disconcerting to the often critical scientific mind, the element of time was thrown in to generate hope--along with the anticipation that some self-assembly features would be forth coming as science advanced. Also, it is true that many biochemical reactions can occur in the absence of a protein enzyme, only slower. With the passing of millions of years maybe time was the element making everything possible. On the negative side if time allowed spontaneous generation it also potentially allowed spontaneous degeneration. Most positive reactions have some rate of reverse reaction. For the degradation of most compounds there is a requirement for oxygen. As stated earlier, the early world was thought not to have much free oxygen. Thus in early evolution, degradation may not have progressed that rapidly. To proceed to the first living cell, all these function would have to occur and be present at the same time and place for the construction of this cell.

There were many other reports of organic compound formation, for example traces of amino acids *have been detected on meteorites coming into the earth's atmosphere* [8]. Thus at that time it was thought possible that somewhere, occurring under some conditions, many of the building block compounds found in biological cells could have been spontaneously developed. Another starting material was carbon dioxide which is currently a component of our atmosphere, but carbon in early geological history existed as the element or in metal carbides. Thus, there was the realization that all the oxygen and carbon dioxide of our planet are the products of living organisms. Photosynthesis which enables the recovery of oxygen from H_2O had not yet evolved. Recovery of oxygen is best accomplished by photosynthesis. Photosynthesis came later in algae, some bacteria and plants in subsequent evolutionary periods.

Dissolution forces

Now the forces of dissolution over long periods of time was troubling to the authors of that era when they state that the point of equilibrium lies far over towards the side of dissolution. " *the spontaneous union, step-by-step, of amino acid units to form a protein has a certain small probability of occurring over a long period of time, however dissolution which occurs at a faster rate is more probable.* "

Carbon and Nitrogen products

Stanley Miller [7] and others set out to demonstrate that some next steps could occur by introducing simple combinations of elements such as C and N that readily form CN (cyanide)

or Ammonium a simple combination of nitrogen and hydrogen. The presence of electrical charge, reducing conditions etc. could, in addition to the traces of a few amino acids, form molecules with resemblances to nucleic acid bases. These are the components of the nucleic acids, RNA or DNA, in living cells. Other physical effects, with other carbon, nitrogen and hydrogen containing entities could cause chemical structures that could be called vitamins, or carbon-containing entities with oil properties. Some oil and water combinations can form circular structures that are reminiscent of cell membranes. There was real optimism that things related to a living cell were starting to come together.

Copy-Cat effect

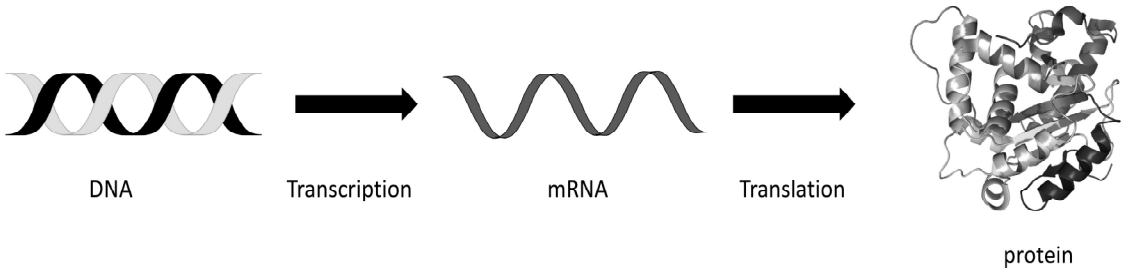
It was also known in the 50s that proteins derived from various biological sources that could self-assemble into hair-like structures that oriented themselves into the formations of structures developed by other identical proteins when seeded into a mixture of the unstructured molecules--a copy cat effect. Maybe such assemblies could occur with simpler molecules.

DNA helix and the triplet code

Watson and Crick [9] discovered the double stranded DNA helix and others [10] found the triplet code of DNA bases A, T, C, or G. These bases, when in a cellular DNA strand, have the code information --transferable to RNA that in turn dictates, through this RNA code intermediation, the composition of proteins. It is these proteins (enzymes) in developed cells that carry out most functions, thus this interconnection has been termed the holy trinity of biology---DNA-RNA-protein.

Holy trinity of biology

DNA → RNA → protein



As noted, DNA contains the code of life, a blue print for everything the cell and eventually for all a whole organism does. The simplest known cell contains 1354 open reading frames equaling 1354 genes [5]. The code is transformed as needed to RNA, with a complex machine, called the ribosomes, that directly assembles the specific protein. Finally, the proteins function, most importantly as enzymes, that perform a multiplicity of functions, from formulating

architectural features of the cell wall and cell membranes to catalyzing metabolic reactions. Again, these enzymes, vital for the cell's/organism's life, are directly constructed from the information provided by a sub-sequence of the total DNA, called an open reading frame or the information in one gene. For an enzyme with 100 amino acids a base code of 300 base pairs in length would be required. In a living cell, the protein produced goes to a cellular location and becomes functional. Eventually each protein in a living cell is specifically removed and recycled. The numbers of these enzymes/proteins for even the simplest cell typically run in size from over ten thousand to over 20 thousand in molecular weight and usually composed of about 100 or more amino acids. Additionally, small proteins, peptides, can likewise be coded and function in vital cell processes.

Defensin is a protein (or a long peptide) [11] containing the following amino acids.

ALSFLFLFLFVAQEIVVTEANTCEHLADTYRGVCFTDASCDDHCKNKAHL-ISGTCHNFKC (The letters represent abbreviations of the 20 amino acids and this highly conserved sequence of amino acids comprises a peptide that operates as a defensive molecule in many plants and animals.)

In the era of the Watson and Crick when the double helix of DNA was determined, there was a less-complex view of the cell construction and function even if there remained gaps. My point is they did not know at that time how big the gaps were. The absence of major missing components was by-passed in thinking that the fill-ins needed only millions of years to formulate the required macromolecules. Having the building blocks: Amino acids, nucleic acid bases and vitamin traces, this would be in line with what a cell would need to form life. However, a little was known about self-assembly, and how helical structures can dictate to other entities compatible helical structures. In this developmental mode molecules might also simply self-assemble into macromolecules such as RNA or single stranded DNA.

It was known that RNA and DNA could link up with hydrogen bonds to match opposite bases on the other molecule, namely A-T and C-G. This base coding property continued through to the RNA molecule except that the RNA molecule utilized another similar base "U" substituting for the "T"base of DNA. The DNA double helix is composed of matching sequences of these bases except that the directionof the DNA backbone must be reversed on the second strand. That is, the first strand direction of the 3', 5' of the phosphate groups (on the ribose backbone that links the bases together) is 5', 3' on the second strand.

Brief view of how the DNA molecule is replicated

It was also evident at this time that the earth had existed a long time and had contained the elements and had experienced the harsh environments reproduced in the lab of Miller. Additionally, traces of amino acids had been found on meterorites so they do exist in space and

thus in the open universe [8].

Connecting dots

Diversity: Even with the obvious diversity of life forms on the planet there was a conservation of some molecular types. For example; the cytochrome function was found to be mostly conserved in post-biotic evolution and the slight deviations in its amino acid sequences were used to follow the route of evolution by various organisms [12,13].

The cytochrome trail through post-biotic evolution

By comparing the sequences of these proteins with other life forms, routes of assembly or variations occurring during species development, could be established. It was found that cytochrome's amino acid sequence was highly conserved in the living cells of all organisms, with no major change throughout the periods of evolution. The tracking with this comparison technique was improved when scientists started using the "ITS" [14] DNA sequence, which was a sequence associated with ribosomal DNA, a DNA needed by higher forms of life. In the intervening years ITS sequences have been used to develop routes of evolution for the vast diversity of life from single-celled creatures to us. All of such data does suggest there was only 1 basic design throughout post-biotic evolution.

Barring many of the steps that most biologists knew were far from being resolved, this analysis was sold as how life was formed--given the massive number of years in which these unresolved events could have occurred and the variable harsh conditions that at one time existed on earth.

Are we about home free without any requirement for an intelligent design?

Was there no intelligent design operating in the conversion of simple molecules such as amino acids, sugars, fats, and ultimately proteins and nucleic acids into the complex structure we know as a living cell? Cytochrome must have been part of an inherent unique design since it was retained so authentically for all of those ages of post-biotic evolution.

On to the route to the living cell

Much needed is some way to devise a pre-biotic route that yields an intact living cell. Well that is the stickler, but let's not worry about that just now, as there are other things that became known that might be subsequently added to this prebiotic puzzle .

As mentioned a major advance came when **Marshall Nirenberg and Henrich J. Mathaei [10]** showed that a triple code of the 4 bases of nucleic acids dictated the order of assembly of amino acids in a protein in a living cell. Unfortunately, we still have to worry about how the associated complex machinery came about that was necessary for the transcription

and translation of this code to the final protein product. Additionally, the resultant function of the protein is based on the orientation of the amino acids within.

Observations of a bystander

As a genetics student (actually plant breeding student) in the Watson-Crick era these advances in our knowledge were impressive and it did indeed seem to me that given time, most of the dots could be connected ----eventually. My interest at that time was to analyze the content of amino acids in plants as they were resisting fungal pathogens, so I was excited about the rudimentary, paper chromatographic technique which was the advancement of that time for separating out all of the amino acids of plant tissue and I got the thrill of being the first to analyze the amino acid content of watermelon tissue [15].

The actual question of my research was, why is one variety of water melon more resistant to a rotting fungal pathogen than another? Was it because of the greater presence of certain amino acids? This study was followed by another assignment to follow the biosynthetic route of how the vitamin nicotinic acid is formed in plants. Biochemical research at that time had almost completed the biosynthetic routes of almost everything in bacteria and a lot of the metabolic routes in higher organisms.

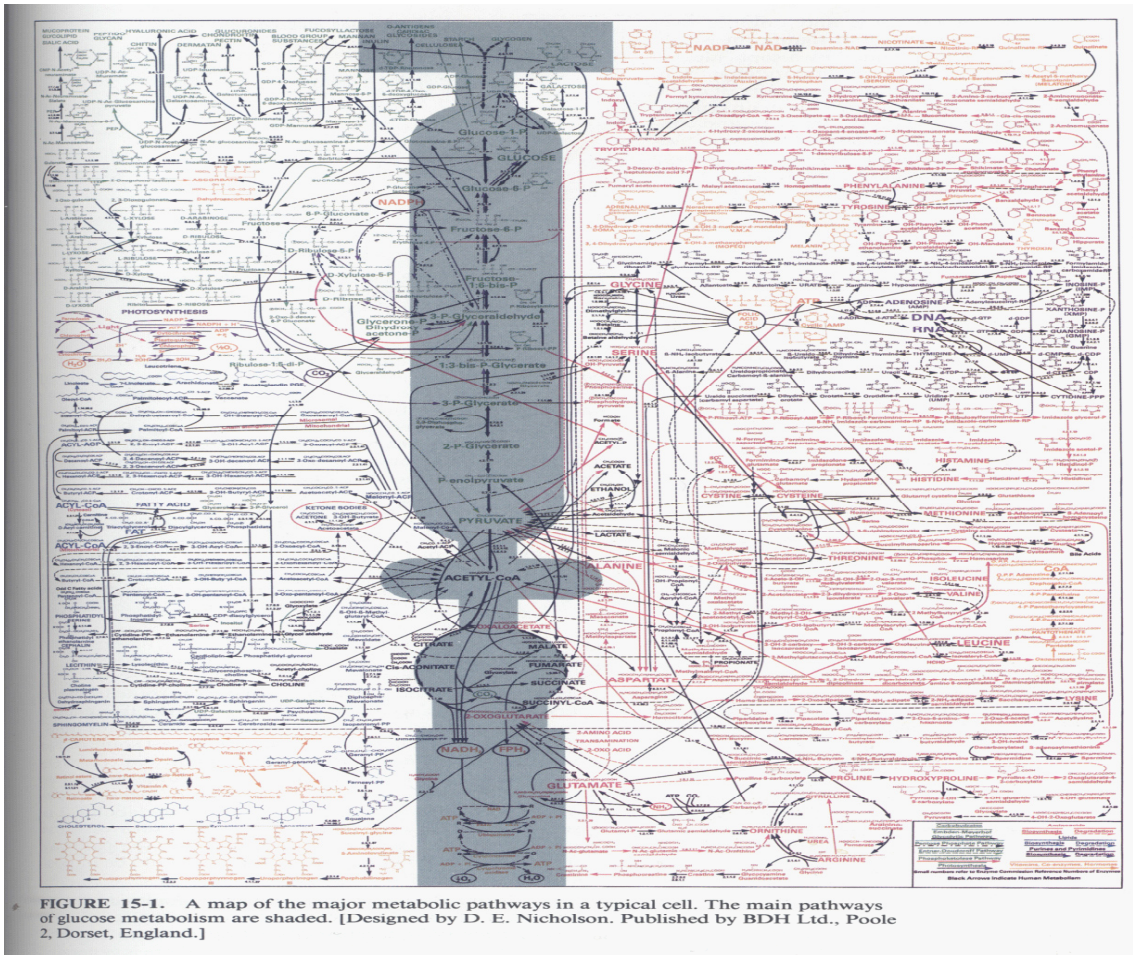


Figure 1: Map of the major metabolic pathways in a typical cell. (Designed by Donald Nicholson. Published by BDH Ltd., Poole 2 Dorset England). Photo from Chapter 16. Introduction to metabolism page 550. Biochemistry V. 1 Voet, D., Voet, J. G. 2004.

Unfortunately, although basic research is always a useful part of the pyramid of knowledge, the amino acid content and the information on other metabolic routes did little at that time to help us understand the mechanism of disease resistance. I remember being chided by a mentor, Thad Pittenger, a *Neurospora* geneticist who allowed me to work in his lab. He said that he could go out the door and grind up grass or a tree leaf and find enough nutrition (from the amino acids they contain) for the watermelon pathogen I was working on to grow profusely, so why would the amino levels be of concern? Yeah, he was right it was something else, and after 50 years of research with the aid of decades of technology the answer is closer [16].

H. G. Floss and I [17] touched on evolution of amino acids in plants at UC-Davis where our mentor, Eric Conn, was into cyanide metabolism. Structurally cyanide looked to be a precursor to a plant amino acid called β-cyanoalanine, which it was, however it needed to add, by enzyme, to a preexisting molecule in plants called L-serine. This process was definitely post-biotic evolution.

I was assigned by another mentor, G. R. Waller to try to take two metabolic steps in plants from nicotinamide to nicotinic acid and N-methylnicotinamide *in vitro*, since this route was evident *in vivo*. This was accomplished, but the second step of the reaction was meager. Both steps relied on the support of an extract from a living cell containing the requisite enzymes and I had supplied all of the known requirements of cofactors [17]. This is when my visualization of how difficult the assembly of hundreds of reactions for the required metabolic pathways-- known to be a part of what any living cell required-- would be to incorporate into the first living cell.

Interestingly, Dowler et al. [19] has more recently devised a prebiotic synthesis of nicotinamide, an importantinput for one part of a metabolic pathway. Additionally, Raffaelli [20] has made a case for the nicotinamide coenzyme synthesis being a by-product of the RNA world [21] arriving at a similar point by a reversal process. Keller indicated the plausibility of a non-enzymatic glycolysis and pentose phosphate pathway in the Aracheran ocean [22].

Amino Acids and their Protein Entities

How many genes does it take to assemble a living cell?

It is really difficult to find a starting place, since the living composition of the simplest cell or organism involves over 1000 genes, in simple organisms, to 20 thousand genes typical for higher organisms. Further, the omission of a handful or sometimes even one of these genes can prove to be terminal for the organism it serves. The gene/protein interdependence is very strong. In the early 40's a Noble prize was awarded to Edward Tatum and George Beadle [23] who discovered that there was one gene designated to each enzyme. This was known as

the "one gene-one enzyme" hypothesis. It later shown to be a bit more complicated but this served as a general rule. This was all worked out in micro-organisms that produced all of their own amino acid requirements from simple nitrogen and carbon compounds. A mutagenic disruption of one of these pathways meant that the organism could not grow properly and the problem could be traced back to the damage to one gene that was responsible for coding one enzyme. The mutant organism did OK when you supplemented the amino acid that was not synthesized, so you could nutritionally repair the omission caused by the mutation.

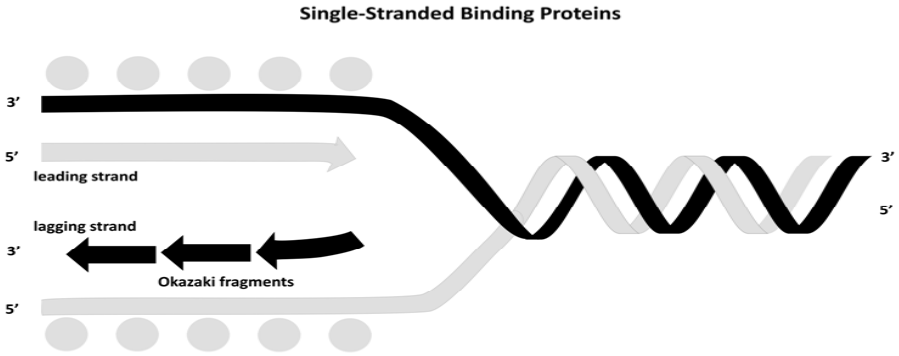
I mentioned previously the historic coding-enlightenments associated with nucleic acids and briefly their need for the requisite machinery to replicate and function. Much of this machinery is also protein based. I will start with very conserved proteins, called histones, that in higher organisms are intimately associated with the cell's DNA. They also help keep the activity of the genes in check –because the cell can't tolerate having all of its genes being transcribed and translated at the same time.

In pea, Cal-Tech's James Bonner and eventually my lab (I hired Chris Sanders from Bonner's lab) worked on the purification and function of histones [24,25].

Histones (I, II, III and IV) are among the many proteins the higher organisms can not do without so they were highly conserved. Which means that they remain chemically almost the very same through evolution, even though present in widely different organisms. That is, histone derived from a calf thymus and one derived from a pea (which I work with) are very similar in their content and sequence of amino acids. In a living cell, the negative charges of the DNA attract the positive charges of the histone and the two molecules are held tightly together. By regulatory design the histones called H2A and H2B get in the way of the machinery noted earlier and have to be temporarily pushed aside to let the transcription complex (the complex that transcribes the DNA information, when needed for a gene product into its RNA form) transfer this information to proceed on to developing a requisite cellular function. In essence, it enables a gene to become activated and generate a protein used within the cell. The transcription complex to exist in the first place has to be pre-developed and posseses a primarily an enzyme, called RNA polymerase [26] (the enzyme that transcribes the DNA message to the RNA message). The "transcription complex" also can associate with about a dozen components called "transcription factors" that make it a suitable machine to type out a given RNA. These transcription factors themselves are also gene products. The transcript complex can be altered in many ways including from signals from smaller molecules like steroids, etc. These signals help (or detract) from the mobility of the transcription complex located on the DNA "railroad track". In front of the "Transcription complex" are the histones and other components. The railroad is not straight -- its DNA exists mostly as what is called supercoiled DNA or DNA with proteins (histones) that are tightly compacted into units called nucleosomes. A general rule is, that the tighter the packing the less able it is for a gene contained within to be

transcribed. The cell has ways to shake loose some of the shackles. It can loosen the super coil by clipping one of the strands of the double stranded DNA. It will unwind like the rubber band in a toy airplane. It can also pull off some of the histones [26] from the track as well as other proteins blocking the way (Some living cells do not have histones and are a bit simpler). It does this by a multiplicity of histone modifications one of which is a mechanism called ubiquitination. Ubiquitin is the angel of death for essentially all cellular proteins (that can be followed by a re-incarnation to new proteins). Ubiquitin latches on to the protein and takes it to another place for destruction. This is often a good way to partially clear the railroad track and allow some selected genes to be transcribed [27,28].

To me it is amazing that the cell has figured a way to recycle all of its proteins and do it as the cell continues to exist and thrive. With my limited comprehension I am unable to understand how all of these controls for each of up to 20 thousand genes and their products could have come about. Moreover, it must have had to occur somewhat simultaneously to assemble the first living cell. These basic steps, as amazing as they seem, are not in my opinion, as amazing as the machinery required to replicate all of the DNA, separate and retain it almost flawlessly to the developing daughter cells. Again an enzyme called DNA polymerase duplicates DNA by assembling bases into the new strand by going in a single direction on one strand and since the matching base on the other strand has its backbone structure going the other way the replication of the other strand must go in spurts synthesizing a few bases at a time and then assembling them again to keep the match of the sequence of the other strand so that the replicated DNA going to each daughter cell is authentic. These little pieces are called Okazaki fragments.



At this point I would like to indicate that this document is not intended to be a course on biochemistry or molecular biology, but a presentation of scientific knowledge that will provide the reader with the actual complexity of the cell and thus the complexity of the life we are witnessing even in the simplest cell. It is important to describe to non-scientists how difficult it is, at least for me, to attribute all pre-biotic evolution to random associations and happenstances.

Most of this information on the **post-biotic** cell can be googled and more can be found in basic biology texts and especially detailed in biochemistry and molecular biology texts. The

true genius of biochemistry text authors have provided detailed descriptions that are easy to follow.

Mutagenesis, the major tool of post-biotic evolution

Now let's go back to the major tool of post-biotic evolution, **mutagenesis**. Knowing that the miracle of the first living cell has happened, no matter how or where, one might easily disregard the importance of the true missing-**pre-biotic** link of evolution. The simplest mutation is caused when the DNA machinery makes a mistake, when one base is not inserted properly and becomes substituted with another base. This change is carried on, since the now new triplet code for the amino acid to be inserted indicates that there may be a different amino acid in the resultant protein. This potentially makes the protein better (or worse) for the organism. This change in DNA can also make it more suitable for getting other genes transcribed. Other alterations include duplication or loss of genetic material. Environmental effects like UV light can have an effect for example. UV light causes the base thymidine (the "T" base) to bond (dimerize with) to another thymidine base, an alteration that the cellular DNA can not tolerate, thus all cells have evolved a system for cutting out this blemish and retyping a replacement strand. In the replacement process the cell can make an error so the sequence is not the same and the effect is again carried along to the final protein product which may become non-functional or in an extremely rare event become more functional, and thus the organism itself can become more fit.

Beneficial mutations

Alterations such as these mutations are capable of the microevolution that most scientists accept, since these types of evolutionary changes can be documented. In higher organisms the Jumping genes discovered by Barbara McClintok [29] can cause alterations in many ways, because these transposable elements can cause duplication of DNA regions or land in the middle of regions that then perform differently afterwards than they did before. These are the mechanisms that probably are the basis for the evolutionary trends in biology observed by Darwin [2].

My point is, there had to be an amazing organization of cellular processes for such mutational events to occur and allow the cell to survive. Without the ability to repair, insert or otherwise remain viable, any resultant beneficial gains would have been very improbable. This "**post-biotic**, **microevolution**"is understandable. In fact it can often be demonstrated in experimental investigations. However, it would have had to occurred using the complicated machinery acquired by the first living cells. The pre-biotic evolution up to the first living cell seems to me to have been both the largest hurdle and demanded the most comprehensive design.

It has always taken a lot of faith to accept that there is some supernatural force that generated the original intelligent design. Who designed the designer? What surprises me is that many scientists read the same literature that I do and yet are reluctant in the absence of knowing how things really came about, to give thought to the possibility that a super-natural entity or God exists. My description of the complexity of biological process just scratches the surface of the complications of biological facts that research is each day uncovering. Of the scientific journals that I read, complete coverage of new biological mechanisms I find are often in Molecular Cell. Along the way the biochemists have done an excellent job in understanding and plotting out all of the steps involved in the metabolism of a living cell seen earlier in **Figure 1**. These charts also show all of the essentials from energy production to the synthesis of carbohydrates, fats, proteins, nuclei acids, polymers needed for cell structure. Once seeing the complexity of these processes, no simple hypothesis is available to explain how the **pre-biotically**-developed cell came about.

Demonstration of micro-evolution or man-manipulated evolution

Some good examples exist for how new forms of an organism can evolve, and more impressively, also be scientifically generated. The wheat we see growing commercially has evolved from some grassy plants. The evolution of wheat can be duplicated by conventional plant breeding techniques that combine different sources of chromosomes. Bread wheat ended up with 3 sets of 7 chromosomes from various sources [30].

Again, this **post-biotic** development is much easier to understand than the **pre-biotic** evolution that starts with elements off the periodic table. Alternately, the possibility of environmental conditions and step wise accumulations of complex structures from traces of amino acids and bases as substrates for reactions can only be imagined from specialized mechanisms incubated in bogs, volcanoes, on rocks, soil, ocean vents, etc,

Multiple pre-biotic hypotheses have been put forth

Since Stanley Miller's [6] time multiple hypotheses have been put forth. Some proposing that RNA rather than DNA was developed first [11]. Other hypotheses implicated proteins in the early developments. Again, the missing answer often given by pre-biotic evolutionists is that all of these things developed incrementally over many years. It certainly would have needed to go incrementally, but the incremental trails are not readily apparent. Surprisingly, the evidence has really not progressed that much from Stanley Miller's time, through an era where the technology associated with molecular biology and genetics, especially mass spectrometry has made major advances. With gene editing the speed of micro evolution may be an important concern for future evolution.

There was a bit of arrogance expressed on the basis of what was known at those earlier

times. Crick the co-discoverer of the DNA double stranded helix, in a visit to Washington State University in l966, proposed making science the true religion for man since all truths would then be derived from experimental data. As mentioned previously, the facts and thinking of many evolutionists were assembled in an Issue of SCIENTIFIC AMERICAN that contained a series of articles by various authors [7]. I would like to present some excerpts from this publication and others that relate this thinking:

Specifics of some hypotheses

As indicated previously, it was easier to imagine the steps in pre-biotic evolution in the 50s -60s than it is now, since we didn't realize how complex things were inside a living cell. The Scientific American authors did recognize and discuss some of the spaces that needed information to fill in between the dots. However, that mind set of scientists of the era has carried over to present days even with the knowledge of how much more the complexity of the simplest cell has been amplified. The feeling remains so strong that any scientist, such as myself, or a biology student that has a problem accepting pre-biotic evolution as being as solid as post-biotic evolution, can be chastised by the scientific community as a non-thinking idiot. Most of the scientists apparently worry less about pre-biotic evolution, as the data supporting micro-evolution beyond the first living cell continues to accumulate. We know more about the many ways genes once developed can utilize the living cell's genome to insert a function or at least exist in the cell. Such genetic manipulations are possible in many and eventually in all higher organisms. The genetic engineering technology of plants takes advantage of a bacterial pathogen of plants that can insert new genes via a plasmid (a little extra piece of DNA found in bacteria) into plants. This plasmid in nature carries DNA into plant genomes, with the information of genes that produce hormones eventually causing tumor-like growths. Molecular biologists such as Eugene Nester [31] revised the plasmid to be capable of carrying a substituted gene of choice and thus the bacterium has become a great tool for the whole world to use for adding genetic information from another source to the plant genome.

Other genetic variations evolving within living cells

Other mind boggling events that occur in living cells, that are difficult to surmise have developed apparently by random events aided by mutations over millions of years.

A back-look at my pea cells

It is difficult for some to think in terms of the following measurements-- but in pea tissue there is a meter length of DNA in each nucleus of each cell. These nuclei are only 10 nm in diameter! Now it takes 30 minutes or so for the cell to divide and distribute 1 copy of the double stranded DNA which is nicely assorted into 7 chromosome pairs to the new daughter cells. These DNA sequences are blue-prints for everything the plant ever needs to do. The

duplicated DNA helixes could be visualized as "two slinkies" scrunched together and then needing to be separated north and south in the next cell division. Evolution has dictated that these new DNA strands do not need to be unraveled to be separated and there is insufficient space and time to do this anyway. Also as mentioned earlier the new DNA strands needs to be re-coated with the usual proteins. For many years those trying to develop models for separating these strands could not make their models conform to what actually happens. Then the enzyme, called Topoisomerase II [32] was found. This remarkable enzyme can cut the DNA and take the two ends around the blocking strand and assemble it on the other side, doing this on hundreds of locations and allowing each of the DNA molecules to by-pass the need to unravel before separation. Topoisomerase II is essential for all eukaryotic organisms, it untangles unknots DNA bypassing an intact helix through a transient double-stranded break that it generates in a separate helix. Thus the whole meter of DNA is routinely handled in this way. Who could have designed such a useful enzyme or have suggested its properties simply happen? This process occurs at cell division and is called "mitosis" in biology texts. (The separations and manipulations of chromosomes in the process of sexual reproduction, called meiosis are equally amazing and as precise). Again, all of the cellular functions of mitosis would have needed to be present in one place and at one time prior to the first living cell.

Another amazing requirement of organismal life is a process call apoptosis. It is not possible to cell-divide the way to becoming an organism. That is, if the cell only knew how to divide and not to differentiate, all life would consist of a glob of cells in a big pile. We now know the process "apoptosis" plays a major part in development by actually terminating daughter cells that do not fit the architectural plans. And of course the surviving daughter cells have to change enough to specialize into leaf, stem root, etc. (or eyes , ears and noses etc. in animals) There are some specialized genes that are called box genes that have major architectural in puts involved with differentiation. These are very specialized in some organisms and can dictate structures such as vertebrae and molting sequences of insects etc. What a design!

Where does increase in complexity end?

It doesn’t end. The more we know about cellular functions, the more complex it all becomes. We know the kind and variety of box genes which are involved in differentiation to get structures from spinal columns to molting events. The genome sequence for man is known, now the genome sequence analysis is possible for each man. “Further the DNA sequences are becoming known for many other microbes, mammal, plants etc. We have the complete *Homo-sapiens* DNA, why can’t we control human genetic diseases or figure out how all these genes work? Some we can. Having the DNA sequence is nice but the complexities of gene regulation and some of the functions of the gene products continue to be undefined. We can analyze functions of some individual genes or groups of genes. We will probably be able to re-develop an extinct mammoth someday using an elephant as a surrogate. The elephant would again provide

the sophisticated machinery to process the mammoth's coding.

What's going on?

In this golden age of science we are steadily finding out new information. The discoveries have contributed to our improved health and even maybe more importantly, they contribute to the abundance of our food supply.

We can live with, and most are OK with, understanding the complexity of **geological systems** developed over time and are mostly open to some alteration of hypotheses as new finds are reported. In general, biological scientists are expected to accept **pre-biotic** evolutionary theory without question? My fresh-out-of-a-biology-class grandson stated, " how can anyone doubt evolution". Of course, I accept post-living cell evolution and don't doubt it. Additionally, I am interested in further research to find additional explanation for how things have developed? Certainly I consider the current documentation of *prebiotic* evolution weak and lacking at best. Pre-biotic evolution should be treated as an open question, one demanding much more substantive research.

An RNA world

Some of this current research thinking about Prebiotic evolution has appeared in journals. For example, a hypothesis appeared that indicates that the ribosome may have been a self-replicating intermediate between the RNA world [21] and cellular life. It is known that rRNA (ribosomal RNA) contains genetic information for encoding self-replicating machinery, namely, all 20 tRNAs (tRNAs carry all amino acids to the site of protein synthesis). Ribosomal proteins themselves when transcribed are also so processed. The authors suggested that rRNA, mRNA, and tRNA had a common ribosomal ancestor [18]. Of course to visualize how the ribosomal system developed constitutes a remaining major gap in understanding this complexity.

Substantial interest and possibly research is **also badly needed** on the concepts of intelligent design. There is no doubt in my mind that what we have documented, so far at the molecular level within a living cell, reeks of intelligent input. At best we need additional time and tools to further define how the intelligence aspect originated. Some advocates of intelligent design avoid the question of who designed the designer? By saying there is no need to fully understand the origin of the designer, except to know that an object was designed. Thus no need to determine if the design originated from outer space, etc. Again the main objective in this book is to bring forward, to those not schooled in biochemistry or molecular biology, a description of how magnificent the cellular design is and of how the many things are contained in the simplest of living cell functions.

Some vital concerns

What are the demands for self assembly: Given an ability for in some manner to have the traces of amino acids, bases or other basic units assemble into a macro molecules, where do you go from there?.

What tricks does the cell need to know? Using the information presented above what is required to develop a plan to for assembling information in a form in which it can be functional, saved and replicated? When assembling:

1. Supplies of bases must be present when the developing cell needs them for assembling them into DNA molecules.

2. All element changing physical forces would have backlogged sugars, amino acids, vitamins, etc.

3. More physical entities such as montmorillonite for nuclear base polymerization.

4. Polymerization and variable heat regimes for strand separation and re-annealing

5. Physical aids for long polymer length and random assortment of bases to give ribozyme-like molecules.

6. A liquid bog that will allow organic reactions to simulate metabolic pathways

7. Discrete pausing steps where partial assemblies can be stored.

8. Bio-processes that assemble membrane-like barriers.

9. Energy development for catalyzing reactions.

10. Partitioning to allow reactions for vital process that might occur to develop in suitable conditions of pH or cofactor concentrations.

11. Combining processes and implementing replicative mechanisms to advance maintenance of functions acquired.

At this point any additions to living cell requirements become just statements without any scientific basis in reality.

Molecular biologists would not be impressed with any coding assembly that didn’t have a DNA start codon like the ATG base sequence and then at the end a stop codon like TGA. Is there some random chance for the sequences to get something functional from this information? This also would require that the sequence information be transferred to “RNA or to some entity unknown currently that could create or store a function". Even the most rudimentary

living cell in existence has the capacity to turn the DNA code to an RNA molecule, namely , RNA polymerase. This enzyme itself is very complex – composed of the 20 plus amino acids that are in the right order to make possible the protein's ability to develop catalytic activity. Looking at a typical cell, this enzyme would be expected to recognize and assemble ribonucleic bases into an RNA molecule with the corresponding base sequence. Now typically this RNA (mRNA) would go to another very complex structure called ribosomes also composed of multiple proteins with specific actions. As indicated above, at this point proteins are assembled with (tRNAs) that deliver the appropriate amino acid as needed to develop a protein product [33,34].

This protein product could then have a function if it possesses the right blue printed amino acid sequence. Again, proteins in cells mainly function as enzymes or structural components of a cell.

The most frugal, simplest, smallest cell then needs 10 enzymes at least to accomplish each of the simplest metabolic tasks [32]. Now all of these enzyme components are fragile and each enzyme as we know it now has specific p^H requirements as well as cofactors that are required at least for optimization of the enzyme's function. Also the processes that it needs to catalyzed has to be at hand. If one looks at a metabolic chart again (**Figure 1**), most of the processes catalyzed within are essential for life. That is, in their absence the living cell is doomed with the omission of simple things such as energy production, transport, chaperone action etc. to get things where they need to go. Each must appear at the right complexity and capacity when they get there to accomplish the mission.

The DNA of prokaryotic organisms, is characteristically without a nuclear membrane, as opposed to a eukaryotic organism that has its DNA mostly within a nuclear membrane. The simplest prokaryotic organism of which I am aware was mentioned earlier. To reiterate, It has the complete biosynthetic pathways for all 22 amino acids.

It has the smallest genome (1,308,759 base pairs). This marine α- proteobacteria (*Pelagibacter ubique*) is found throughout all oceans [35]. The smallest free-living eukaryote is *Ostreococcus tauri* and has a 12.56 Mb (millibase) nuclear genome [36]. It has many metabolic pathways including genes for the photosynthesis apparatus and others-- possibly for C4 photosynthesis.

Further evolutionary requirements we can rationalize and some we all can understand

1. All of the factors must be regulated as most folks taking medications know. The living cell has also determined that components much be recycled. Believe it or not, our proteins are continuously synthesized and dissembled.

2. All of the informational molecules must be replicated.

3. All other things like substrates and products must be kept at the ready.

4. For any future for the living cell --it must divide.

To be a valid evolutionist you should know biochemistry, at least the biochemistry that the previous paragraphs are based upon.

Obviously all PhD biochemists do understand these complexities of a living cell. And when pressed on their views of the origins of life can again employ the *sandwich theory*. Biology needs an organized starting point. If not via prebiotic evolution on earth there are suggestions that the earth was visited by early alien organisms that left behind biological organization in the remnants of their picnic lunch (ie, sandwich) which contained the first living cells. These cells would have then been seed for the future evolution that could have the millions of years to evolve into all the magnificent creatures we know now exist. This simply moves the problem of assembling a living cell to some other location in the universe.

Micro-technologically can now trace evolutionary histories

The DNA based information that is associated with the ribosomal DNA that was mentioned earlier has been useful to evolutionists. This information within ribosomal DNA would have had to be conserved as it enabled a vital function. Thus some sequences of the ITS region of the ribosomal region of DNA mentioned above have been used as a way of tracing changes within these sequences that would give clues to the routes of evolution that occurred. Using this and other similar molecular tools one can follow incremental changes that occur in this DNA material as one traces family ties as a history of the progression of mutational changes that have occurred. This data enables scientists to develop trees –called dendrogram to understand the paths of diversities. This tracing probably is and will continue to be assisted by the total information in complete DNA sequences of each species. With adequate research all organisms could fit into these schemes. The other maps should be completed to follow many other sequences that are somewhat conserved that may indicate that all of the beneficial changes in proteins that occurred, followed alterations caused by mutagenesis.

Sophistication of benefits

Evolution by alteration of DNA is the name of the change game but most mutations are negatives. So how can the cell retain the sophistications that are really beneficial.

Most eukaryotic cells have about 20,000 genes to regulate, so that just the right genes are utilized for the required processes. *Pelagibacter ubique* has 1,354 open reading frames and thus the lowest no (1,354 genes). Some of the genes are stowed away in their location on

a chromosome. Sometimes they are not readily accessible to the RNA polymerase described above. Sometimes the RNA polymerase is right where it should be to read off the gene's information, but is is "paused" until some special reorganization of that segment of the chromosome takes place [38].

Remember the mention of recycling cellular components? Some of the components are proteins such as the histones, two of which, must be temporarily displaced (from the parading grounds near the genes's start site) to allow the RNA polymerase to read through. These histones are subject to so many changes that commercial biotech companies have a brisk business just developing antisera against all of these modifications to sell to researchers so that they can follow these changes. Likewise these histone-road-bumps can be removed by a process called ubiquitination where a ubiquitin molecule clams onto a histone and carries it off to a destruction area. Sometimes a gene can be activated simply by the damage occurring to the region of the chromosome where it exists [37]. This damage can be radiation from a UV light (via the thymidine dimerization mentioned above) such as that which unleashes the genes involved with melanin accumulation in a process we know as sun-tanning.

An article in the journal MOLECULAR CELL reports that gene control features such as the pausing described above, counteracts DNA and subsequently influences the nucleosome region in the chromosome organization that then allows a gene(s) to become active [38].

All of these mechanisms are required to enable precise gene activation in response to stimuli. There are still other "checkpoint" proteins that respond to DNA damage by halting the cell cycle until the damage is repaired [37]. Thus these examples are but a few of the abundant regulatory features of a living cell. Evolution beyond the first living cell may register as being pretty straight forward to the casual biologist. Simply cause multiple (1000 plus) mutational events and expect that one of these might be beneficial to the organism. This could enable the individual to out-live or out compete its siblings adding a new gene to the evolutionary process. However it seems un-conscionable to me that people who research the sophistication of these events would still not visualize it as occurring on top of an extremely intelligently designed cell.

Examples of Sophistication

The following sophistication examples added to point out what I am talking about.

Insects: Most of us have been impressed with the diversity of insects we run into on a daily basis. I was fortunate enough to see the absolute diversity of a career entomologist's photo collection at a national meeting. Talk about intelligent design. The imagination of all the scripts of scientific fiction movies could never match this. Although we are progressing towards developing sophisticated nano-robots that will function like living entities in our blood vessels.

Alternately, some of the tiny sophisticated organisms that currently exist, such as the bed infesting mites and spiders are so small they often can't be observed with the naked eye.

Yet they are capable to walk, poop, multiply eat dead skin, survive environmental stress etc. With all of this, they have the full complement of all the regulatory features talked about above. This is also true for bacteria and fungi that have spores or cells that are not visible to the naked eye.

Even though scientists are knowledgeable in many areas, one has to realize that each has his/her own expertise. When departing their area of training can become vulnerable to the lack of background knowledge in other areas. This could explain my situation. I am not an evolutionary scientist, but I have tried to keep abreast of the accomplishments as I pursued my chosen field. To be exuberant about future research upon which there is little basis to be exuberant, does not make sense to me. I was listening to NOVA and at the end of the hour, the moderator came out and proclaimed that we would go to other worlds when conditions deteriorate on earth. In opposition other less-exuberant space-investigating scientists provide simple mathematic realities that indicate that even if we attain travel at the speed of light, there are no inhabitable planets that we could reach and if we could develop the amount of energy to get a couple space ships there, the amount of energy required is massive. There is no current possibility for transporting thousands of people, let alone, larger populations to outer space. There is another group of scientists that talk about life being possible on Mars. It is hard to believe they really believe this themselves and probably are associated in some way with NASA. The evidence that life exists (or even existed) on Mars is that traces of water appear to be present and that meteorites of Mars origin falling to Earth have had traces of a couple of amino acids. Additionally, to think prebiotic evolution to a living cell occurred on Mars considering the complication of a requisite living cell assembly in such a harsh environment its beyond my mind.

One has to be discouraged that advances toward assembly of a living cell have not been that impressive since the time of Stanley Miller. It is probably evident to the reader that I am confident, on the basis of the data in the literature that the "micro" incremental changes we see continue to be possible and have occurred. Given the challenges incorporating the properties of elements into the properties of a living cell it may be gentlemanly to at least give some probability that some super-natural power exists in the universe. Some scientists have suggested that those who believe in a higher power can slow scientific advancement. The ideal for the scientist then must be atheism. This is in opposition to reality, because many of the advances in science have been accomplished by scientists that believe in God. Just like there are many unknowns that stand in the way of formulating travel and habitation of planets, there are many unknowns remaining to even conceptualize how the prebiotic evolutionary events lead to the first living cell. My objective with this some what superficial look at cellular complexities is

to ask serious scientists and others to stand back and realize that pre-biotic evolution has some big gaps. I hope to reduce somewhat the condescending attitude the scientific community can portray to pre-biotic evolution questioners when the data on pre-biotic evolution is very incomplete. Theologians when needing to fill holes can refer to them as miracles, scientists might admit we don't know how we got here and be content that someday we may know more. Thad Pittenger an atheist, but a mentor I really admired, when talking about the "here after" indicated that since we knew so much in 1960 that within his life time we will become immortal and live on forever. This man was older than I and survived WW II but like us all, did not attain immortality (Nice thought, but wouldn't immortality really kill the social security system). Everyone should have to decide for themselves how we got here but no one can deny that what we see around us is fantastic and relates strongly of some manner of intelligent design. But what is disappointing to me is that the scientific community remains to discriminate against those who are not dyed-in-the- wool believers in evolution and in a way reminiscent of those evangelistic groups also resist inserting what we do know about evolution into educational programs. This should be a period of open thinking.

The evangelicals cannot and will not, long be able to realistically ignor the data on the age of the earth and the geological periods that lay out the fossil records that are orders of magnitude in contrasts to biblical records. Also to what can be proved by carbon dating and other strategies. Mankind does need to have codes of morality that provide a route for happiness and civilized treatment of his fellow man. Most religions advocate these values. Our constitution is based on these principles. Man undoubtedly could come to know what is basically right or wrong even without religion, but religion has provided some very meaningful lessons that are beneficial to almost everyone. There probably is a case to be made that individuals that never interacted with any religious education potentially are more troublesome to society because of a lack of it. Those who work with rehabilitating prisoners or those trying to help alcoholics or drug addicts recover often are assisted when the individual comes to recognize a higher divine authority. I doubt that the threat of incarceration or the after-life of heaven/ hell are the major motivators of human actions, but surely are bound to enhance some scruples. I was impressed hearing the life story of Michael Caine whose fame was massive and his success rate phenomenal but his life was in line for complete destruction with drugs and alcohol - who in the end was able to recover by his change from addiction to religion. May be this was all psychosomatic but may be not.

Other proposals toward connecting the dots within pre-biotic evolution

The treatment of the topic of pre-biotic evolution would be incomplete without the submission of some additional information inputs that propose potential routes toward the living cell. The current way to become an expert in any field is through the internet. The internet provides an array of contributions toward filling the dots and spaces of pre-biotic evolution.

Unfortunately, the spaces are overwhelming even if there has been 4 billion years for chance occurrences and self-assembly.

Some of the scientific community have delved into proposing mechanisms for how the first cell was assembled and some are peer reviewed and occur in journals. Some journals specialize in pre-biotic evolution however the source of much of the following is presented under the category of ABIOGENESIS and contained within *Wikipedia, the free encyclopedia.*

Since this report is not a review article per se I will rely on this Wikipedia source to list some of the current models. To assure that this is not an exercise in plagiarism I will italicize the information taken directly from the Wikipedia site.

First there is still no standard model for the origin of life

That the first cells were synthesized under natural conditions by a slow process of molecular evolution, would be an expected occurrence, but the devil is in the details. What were the conditions and what molecules were involved? Earth was first thought to have been chemically reducing in nature with materials such as methane (CH_4), ammonia (NH_3), water, hydrogen sulfide, carbon dioxide, carbon monoxide and phosphate. Molecular oxygen and ozone would have been rare. Later models nitrogen and carbon dioxide were thought prominent with some carbon dioxide and sulfur components. There have been examples of the chemical origin of organic molecules, which is nice in its self but a long way from assembly of a living cell. No one, given all of the currently available biological materials, is close to having a complete plan for how a cell was constructed.

Analysis of life forming units by minimization

Other researchers have tried to understand pre-biotic development by taking a living entity and minimizing its attributes to determine what it can give up and still live, thus seeing development from the top down. Having worked with mutagenesis of pea and some with pathogenic fungi, I propose that this deletion process does not get you far. With fungi one can wipe out a biosynthetic pathway for the production of one of the essential amino acids and because of the organism's ability to "transaminate" (a way of transferring parts of one amino acid to another) can live by finding another route for making up the deficiency. In pea plants mutagenesis can alter the DNA in multiple places and the plant often can grow poorly and survive, yes and at a low percentage of the time acquire some beneficial traits or as in plants overcoming the metabolic block caused by the active ingredient in the herbicide, "Round-up". The bottom line is that a living organism has to co-retain a massive base of operation that is still magnitudes of levels above the assembly level needed for developing a first living cell.

If it is not possible using the simple elements or trace quantities of sugars/amino acids

and organic compounds potentially capable of mimicking short reaction runs resembling those of a living cell, the cop-out alternative is again to attribute the first living cell on earth to alien drop-offs. That is, attribute its origin to an outside extraterrestrial source.

Deep Sea vent hypothesis

Hydro thermal alkaline vents occur in the ocean and provide data for another hypothesis for the origin of life. FeS and NiS can catalyse the synthesis of the acetyl-methylsulphide from carbon monoxide and methylsulphide. There is an indication that such pre-biotic syntheses can occur at the inner surfaces of these vents [40].

The surfaces of mineral particles inside the vents have catalytic properties similar to enzymes and are able to create simple organic molecules such as methanol, formic acid, acetic acid and pyruvic acid out of the dissolved CO_2 in the water [41].

Clay hypothesis

One form of clay is Montmorillonite is a catalyst for the polymerization of RNA and for the formation of membranes from lipids. The clay hypothesis suggests *that complex organic molecules arose on a pre-existing non-organic replication surfaces of silicated crystals in solution [40].*

Although the demonstration of polymerization of RNA bases indicates only that- given the bases- the Montmorillonite acts as a catalyst, it is probably one of the most encouraging demonstrations of non-enzymatic formation of organic polymers [22].

Self-assembly of RNA

An interesting supplement to the early role of RNA was suggested by the paper by Stan Palasek [43].

His investigation indicates that the hydrothermal vents found in the ocean could develop variations in temperature that might function with RNA polymers in a manner similar to the Polymer-chain-reaction (PCR) action on DNA. The well-used PCR reaction amplifies traces of DNA to develop forensic evidence and a list of other health related assays known by the general public. The PCR assay works in the presence of a DNA polymerase enzyme and a pool of requisite bases. The role of temperature changes is to melt the DNA strand to enable the polymerase to replicate the DNA and progress in each temperature cycle to increasing the amount DNA and the decrease in temperature allows the strands to re-anneal. The validity of this approach wherein RNA itself can be shown in a live cell situation to be involved in self-splicing. This RNA now referred to as a "ribozyme" was originally discovered in some species of the protozoa, *Tetrahymena* [44].

The demonstration that a RNA replicating property in some cases may substitute for the Polymerase enzyme of the PCR. Palasek **is credited with showing that self-assembly of ribonucleic acid (RNA) molecules can occur spontaneously due to physical factors in hydrothermal vents.**

Thomas Cech [43] has clarified that there are two worlds, a primordial RNA world, the hypothetical era RNA where the RNA served both information and function, both genotype and phenotype and the RNA world of today's biological systems.

Extraterrestrial sources of organic compounds

Some encouragement for biological "building blocks" also comes from the more recent detection of an array of extraterrestrial organic molecules. The reports of these molecules are nicely summarized under the heading of "**extra terrestrial organic molecules**" following the internet accessible topic "**Abiogenesis" from Wikipedia, the free encyclopedia.** For simplicity, in the current document, it is sufficient to list names of the compounds discussed. A subtopic is termed Panspermia, is a term that has been designated for the hypothesis that *life exists throughout the universe, distributed by meteroids, asteroids, comets, planetoids and by space craft in the form of unintended contamination by microorganism.* To innumerate: Hydrogen, helium oxygen and carbon are the most abundant elements in space. Compounds composed of these elements are *relative common in space.*

Ionizing radiation is largely responsible for converting the elements into more complex compounds and ending up in cosmic dust and elsewhere. The following space-detected compounds are components of biological entities: Formamide, xanthine, adenine, guanine, mixed aromatic-aliphatic structures, glycolaldehyde, glycine and some other amino acids, pyrimidine, (uracil cytosine thymine can be synthesized in laboratories under space conditions), polycyclic aromatic hydrocarbons. One has to congratulate the scientists along with their increased technical sophistication for detecting these molecules. There **has been** advances in the era, however, given and abundance of the proper quantities of these and an abundance of physical forces into the mix it remains a phenomenal distance from these minor synthetic processes to the workings of a living cell.

I believe it is appropriate at this point to move from the biochemistry, genetics and physics to the sociological. It is easy using these disciplines to criticize creation as described in the bible or in the many tribes and cultures throughout human history. What must be realized is that human values have been maintained (or reduced) by all of the religious beliefs of mankind. Most obvious to Americans is that our constitution and bill of rights were strongly and positively influenced by religious values. What I object to is how what is known about evolution is often used as a tool to minimize what religion can contribute to a good life and even law and order in our society. This E-book was an effort to point out that evolution is currently an

imperfect tool, lacking the prebiotic link, and it is premature to state there is no higher force involved in what we see around us on this magnificent planet or for that matter in the universe. Also that if a person, scientist or other, is involved with a religion, it should not be considered a trait of ignorance. Alternately, to attribute the micro-evolution documented from the time of Darwin as an indication that it explains all of creation while ignoring the missing link of pre-biotic evolution is fraudulent science. Science should always seek the truth. To spell it out, a professor of biology teaching to a college freshman may persuasively convince the student that Darwinism explains all. By ignoring the loose end of missing documentation of pre-biotic evolution there is a new generation of people abandoning what religion has to offer beyond marrying and burying.

In my opinion, every man in the street would benefit from a course in biochemistry or even a glance at the complexity of things such as intermediate metabolism as seen in Figure 1. The intelligent design obvious, at least to me-- again in the workings of enzymes like topoisomerase II that separates the DNA code to daughter cells by precisely cutting and re-splicing the genetic information of DNA. The continual recycling of all the cell proteins while the organism continues to live. The clotting of blood that is a multiple step process. The workings of the human brain. The preservation of genetic material in salmon sperm by removing histones and replacing them with protamine and eventually re-acquiring histones. The finalization of active growth in the plant with seed formation, followed by the opportunity to re-construct the entire plant when conditions are right. The absolute control of the use of genes only when making a leaf tendril, pedal, or root. The ability of an organism to monitor the very frequent damage to its own DNA and subsequently remove and re-splice the blemish. If these and many more "miracles of life" cannot be categorized as having been intelligently designed, the definition of this term would have to be changed.

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