# Origin of Life: Another Step Forward

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## Introduction

The origin of life remains among the most intriguing, baffling, and mysterious unresolved dilemmas facing mankind. We all experience the reality of life and observe it arising all around us. But it always arises from other similar slightly non-identical parental living organisms. Spontaneous generation has been scientifically excluded as a possible origin of life. We also regularly observe its sudden inevitable cessation or death.

But what really is this phenomenon we commonly refer to as life? Wikipedia provides a ten-page definition of life. The following brief summarization is based on its generally recognized properties discovered by the biological life sciences:

- Anatomy, the study of form at the visible level,
- Ultrastructure, the study of form at the microscopic level,
- Physiology, the study of function,
- Molecular biology and Biochemistry, the study of form and function at chemical levels,
- Ecology, the study of the relations of organisms with their environments,
- Taxonomy, the naming, identifying and classifying of organisms,
- Ethology, the study of animal behavior and,
- Sociobiology, the study of social behavior of individuals and groups of organisms.

The weight or mass of an organism following the cessation of life remains unchanged. Yet all signs of organized physiological and chemical reactions suddenly cease and disintegration of the millions of billions of organic molecules begins immediately. Suddenly it is as if all the switches on the trillions of nano-machines have turned off; and they all start to fall apart. The spark of life is mysteriously extinguished.

It is difficult to keep pace with the rapid progress in scientific understanding of how living organisms function, how they acquire and utilize the raw

materials required to support life, how cell division is initiated in single celled organisms such as E. coli bacteria and the complex, multistage, reproductive processes in multi-cellular organisms. We are also getting glimpses into the physiological and biochemical basis for higher mind functions such as psychological, sociological, and philosophical reactions and adjustments which we all habitually perform. We now have tools capable of observing fundamental life processes as they occur at the molecular level. We can accurately image living molecular activity at resolutions in the range of nanometers or less. But we remain largely stymied in our scientific search for the origin of life (OOL). And in the past 50 years we've gained little if any scientific understanding of the inherent human possession of self-consciousness and the even more intriguing and closely related concept of consciousness of self-consciousness.

Of the many questions that might be considered pertaining to the OOL, we will concentrate on the following three: **First**, what scientific avenues are available in our search for the OOL, an event that apparently occurred once, giving rise to a single evolutionary family tree, based on a DNA or RNA source containing an immense genetic code capable of giving rise to all the interrelated forms of life known? **Secondly**, what is the statistical probability of random, simple chemistry generating a double helical biochemical molecule, totaling more than three billion base pairs in length **encoding** a biological encyclopedic instruction manual for building and operating a living organism consisting of approximately 37.2 trillion cells (in the average human) that work in unison, with a sense of purpose, a feeling of selfhood, ability to relate with other similar biological organisms as unique individual persons capable of expressing love, goodwill, cooperation and an onboard recording system that can record and recall all the important events of a lifetime? **Thirdly,** what scientific evidence is there to support a gradual progressive march toward increasing complexity of cells leading to the synthesis of on average 10,000 protein molecules, averaging 400 amino acid molecules in a precise sequence, in which there are 1 million exact copies of each protein molecule, all continuously functioning in the average cell?

And then there are the genomes of all the other living organisms on our planet both past and present, including the presently recognized four kingdoms of living organisms, Prokaryotes, Eukaryotes, the Botanical Kingdom, and the Animal kingdom.

**First, what scientific avenues are available in our search for the OOL, an event that apparently occurred once, giving rise to a single evolutionary family tree, based on a DNA or RNA source containing an immense genetic code capable of giving rise to all the interrelated forms of life known?**

The idea of launching a scientific search for the OOL by way of chemistry emerged as a result of the Oparin-Haldane Theory set forth independently by Aleksandr Oparin, a Russian biochemist and J. D. F. Haldane, a British scientist in 1923. Their theory, known as Abiogenesis, proposed that conditions present on early Earth were conducive to the formation of organic compounds as a result of spontaneous simple chemical reactions between the inorganic elements and compounds such as ammonia, water vapor, hydrogen, and methane presumed to have been present on early Earth. Oparin considered coacervates, which were suspended colloidal particles in the primordial soup, to have played a prominent role in the abiogenesis process. We now know that they are essentially irrelevant in organic chemistry.

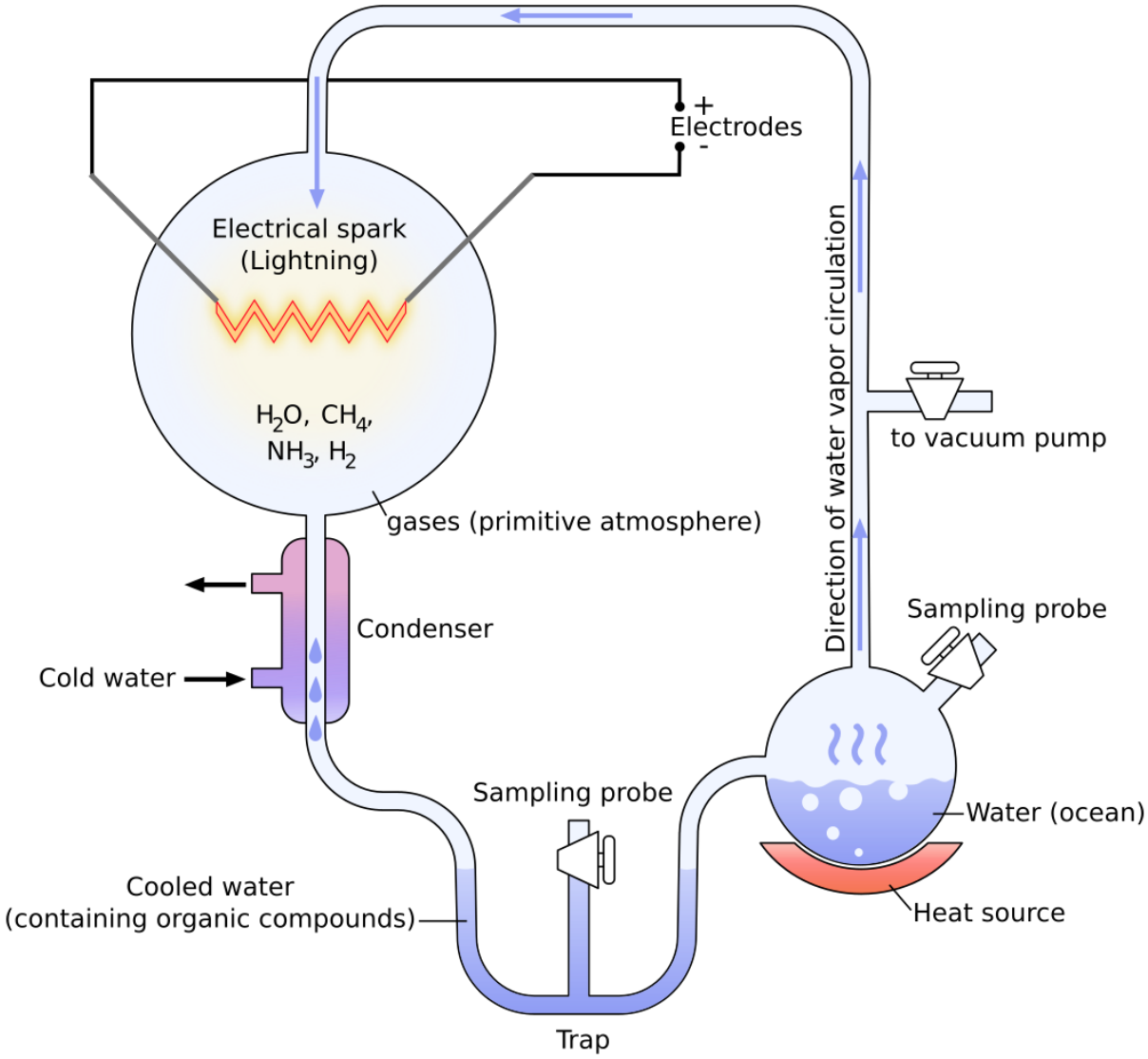
It should be noted that abiogenesis is distinct from spontaneous generation thought to have been the OOL from the 17th into the 19th centuries when it was scientifically disproved by Pasteur. Abiogenesis presupposes the basic processes of inorganic chemistry, leading to organic chemistry and advanced prebiotic chemistry that in turn leads to simple biology. Abiogenesis remains a theory having been neither proven nor disproven.

The first scientific experiment designed to support abiogenesis occurred three decades later in 1953, performed by American chemists Harold C. Urey and Stanley Miller here at the University of Chicago. Their experiment was simple, consisting of a series of flasks connected by a tube forming a circuit. One flask contained warm water, ammonia, methane, and molecular hydrogen, all of which were thought to be present in the environment of early Earth. The water in the flask was heated gently to mimic the warm inland bays and lagoons. A second flask was connected by glass tubing was rigged with tungsten electrodes. Electrical sparks were generated between the electrodes simulating lightning strikes producing energy to stimulate chemical reactions.

The experiment was run continuously for a full week at which time the solution became brown and the flasks became coated with a black oily tar

like substance. Analysis of the fluid contents yielded several amino acids including glycine and alanine and traces of additional amino acids. Other organic compounds included aspartic and glutamic acid. Other experiments followed, yielding additional organic substances. Two decades later a box containing a number of Miller's vials, were found that had never been analyzed.[1] These had been carefully labelled and preserved with direct references to Miller's laboratory notes. Utilizing modern analytic equipment at that time resulted in identifying a significant number of additional amino acids such that more than half of the twenty basic amino acids required for life had been produced.

**Figure 1. Miller- Urey Experiment Apparatus 1953**




The results of these experiments launched the new field of "prebiotic chemistry" captivating the imagination of many OOL scientists resulting in many attempts to generate additional organic compounds found in living organisms. There was considerable optimism and speculation that the Miller-Urey experiment had opened a prebiotic metabolic pathway leading through simple chemistry to simple biology. Although additional organic compounds were generated by further similar experiments, there continued to be absence of a vast number of the most crucial substances particularly sugars

required for life. This avenue of research has been largely abandoned by present-day OOL scientists.

## The RNA World Theory

The history of the RNA World Theory actually goes back to the 1960s at which time Lesley Orgel and others, based on their observance of folding properties of transfer RNA (tRNA) predicted that RNA may have played a major role in the OOL.[2] But it was the work of Sidney Altman and Thomas R. Cech, with their discovery of the "catalytic properties of RNA," for which they were awarded the Nobel Prize in chemistry in 1989, that provided biochemical evidence to support this theory. The potential of self-replication when added to an **information molecule**, greatly enhanced the likelihood of its association with early life.

When one considers that virtually all forms of life depend on DNA to encode their genetic information, and that the encoding system is universally functional throughout all living forms, one cannot escape the conclusion that the origin of life is most certainly linked to the source of information. Based on phylogenetic considerations alone, searching for the origin of life among the information molecules is clearly a step forward from the Miller-Urey search following the metabolic pathway.

All living cells contain DNA and RNA, which are complex organic molecules now generally recognized as **information molecules** because they carry a comprehensive "biochemical blueprint" including detailed directives for synthesizing each of the millions of essential proteins, their precise elemental sequence and structural arrangement, and their exact intracellular relationships composing the millions of unique cells that perform a myriad of living functions, including acquiring nourishment, directing growth, genetic replication, species reproduction, and interacting and adapting to the environment. In short, they control all living activities carried on by the estimated 10 to 30 million different species currently existing on the planet, and these are thought to represent less than 10% of the total number of species that have existed on the planet throughout evolutionary history. The encyclopedic genetic information that is present in the DNA of every living cell, is safeguarded, replicated, stored, and transmitted from generation to generation by DNA **replication**.

Both Altman and Cech were working on the genetic code in the 1970's. They were studying how DNA was transcribed into RNA, a process that required the removal of strands of RNA called introns. Introns are segments of DNA that do not carry information required for protein synthesis, and are

therefore removed, actually cut out, from the RNA strand and the cut ends are then spliced together by enzymatic activity, before the RNA can actually direct the synthesis of a protein by a ribosome. In the past introns were considered to be redundant to the genetic code and was referred to as "junk DNA". Recently, it has been learned that they play a significant regulatory function or control over gene expression.

Altman and Cech made their discoveries independently. Altman was studying an RNA cutting enzyme from bacterium *Escherichia coli*. He found that the enzyme he was studying, RNAs P, a complex between a protein molecule and an RNA molecule, was no longer functional if the RNA molecule was separated from the protein. By adding the RNA, function was restored. Furthermore, he found that the RNA molecule by itself was capable of splitting as well as rejoining itself. This was the first recognized example of a non-protein molecule capable of enzymatic activity.

Cech, in 1982, was studying the splicing of RNA, also in a single celled organism, called *Tetrahymena thermophilia,* when he quite by chance discovered that RNA was capable of cutting itself into segments and rejoining the pieces.

It is well recognized that DNA is an "information molecule." Its information is stored in code form. Each DNA molecule consists of two long strands of deoxynucleic acid molecules arranged in a double helix. The double helical configuration was first proposed by James D. Watson and Francis H. C. Crick in 1952 – 1953 and is now recognized as one of the most magnificently constructed molecules to occur in all of nature. The two strands are complementary such that wherever one strand has an A, the other strand has a T, and wherever one strand has a C, the other strand has a G; this complementarity construction contributes greatly to the stability of the DNA molecule. If the two complementary strands are separated, they will spontaneously zip back together under physiological temperatures and electrolyte concentrations. Its stability is further illustrated by the fact that it can be recovered and accurately sequenced from bones and tissues that are thousands of years old, as was recently reported to be at least 8100 years old in remains that "were not even stored in ideal conditions the entire time."[3]

The RNA World is the continuation of the search for a random process based on simple chemistry that originated the process we call life. It is a

continuation of the attempt to establish the theory of abiogenesis begun by Miller and Urey in 1953, that has now been largely abandoned by OOL scientists. But it is a most reasonable step along the phylogenetic biochemical pathway based on our present understanding of the role of DNA and RNA as informational sources providing directives for the synthesis of all living organisms beginning with embryonic development and extending throughout an entire lifetime.

## Stereoisomers

A major problem with inorganic spontaneous chemical reactions as a source of protein building blocks for living organisms is the fact that functional living protein molecules are almost all stereospecific. In life all amino acids, for example, are left-handed stereoisomers and sugar molecules are all right-handed stereoisomers. A major insurmountable problem occurs because natural spontaneous prebiotic chemical reactions producing biochemical molecules always produce mixtures that are roughly half right-handed and half left-handed isomers known as **racemic mixtures**. Stereoisomeric specificity is a hallmark of life and is one of the mysteries for which no explanation has been found, and no solution has been put forward by the OOL scientists who are promoting prebiotic chemistry as possible pathway to the origin of life.

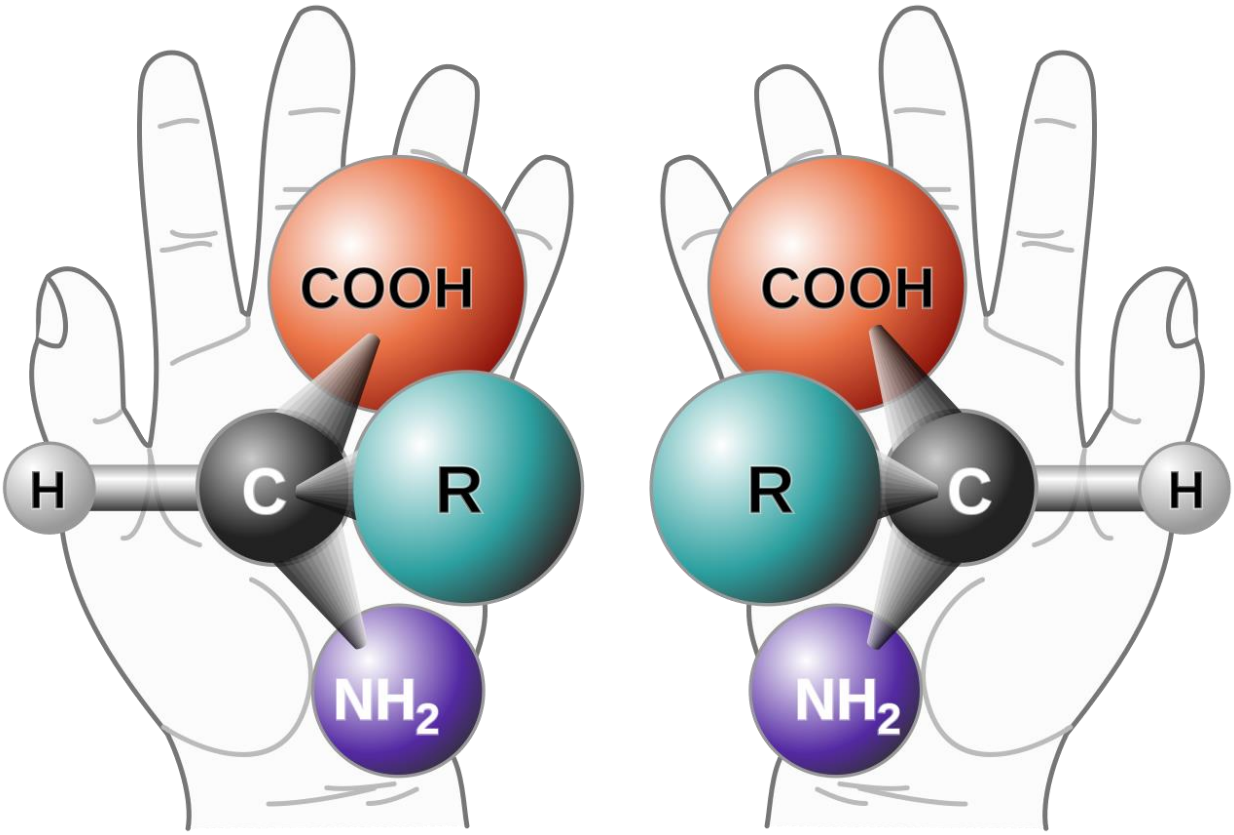
A **catalyst** is a substance that can facilitate or potentiate a chemical reaction without itself actually being changed or consumed in the process. In nonbiological chemical reactions, catalysts function primarily by attracting atoms and molecules closer together so that the reaction between them occurs more rapidly than it would otherwise. The reactions are still dependent on basic chemical forces that form covalent bonds, and the much weaker noncovalent bonds such as hydrogen bonds, Van der Waals interactions and ionic attractions. In contrast, catalytic action of **enzymes** in living organisms is much like a series of robots performing specific actions in organizing and arranging the molecular bonding process. Enzymatic action is characterized by speed, precision, and specificity, including stereoisomeric specificity.

Stereoisomers represents a commonly recognized phenomena in living organisms in which two compounds with identical molecular formulae occur

in different spatial configurations. The atoms in the paired stereoisomers are all linked together in the same order, with identical bonds however the 3D spatial arrangements are mirror images of one another. They are generally referred to as right-handed or left-handed that are designated by the letters D and L respectively.

Stereoisomers, also referred to as **chirality**, is a frequent problem in biochemistry because carbon chains commonly form the spine of polymers that make up most proteins. The carbon atom has four possible covalent bonds to which a large variety of other atoms and radicals can attach. If all four of the attached moieties are different from one another, then a chiral center exists in which the possibility of two isomers, one left-handed and the other right-handed can occur. The simplest way to conceptualize this is to look at your two hands. Both have four fingers and a thumb, in each the thumb and index finger oppose one another, both have extensive grasping capabilities and a high degree of dexterity but their spatial configurations are different and fit very differently into small confined spaces. The left hand does not fit into a right-handed glove nor vice versa.

**Figure 2. Stereoisomers-Mirror Images**




In all nonliving chemical reactions such as the Miller-Urey Experiment, when amino acids are produced they are a racemic mixture.[4]

But the fact that only half of the compounds produced can be utilized is a minor factor compared to the problem where stereoisomers of the amino acids are all mixed together so that the polymerization reactions that follow, will result in a string of amino acids in which the left-handed and right-handed amino acids will be randomly mixed resulting in nonfunctional or potentially toxic molecules, incompatible with life. Since, so many chemical reactions in living organisms involve molecules fitting together like a lock and key, spatial configuration is a major factor.

Stereochemistry is a major branch of chemistry within the pharmaceutical industry. Because of the structural differences depicted in the mirrored images of a molecule, each stereoisomer usually has different biological activities because of the different spatial configuration of atoms within their structures. Thus, their ability to interact with other molecules differs.[15]

## Stereoisomers and Pharmacology

The first step in this process involves determining whether a **stereogenic center** or **chiral center** exists within the compound. "If a carbon atom is bonded to four different atoms or groups, it is a chiral center, and the molecule has a non-superimposable mirror image."[5] Most molecules isolated from living organisms are chiral and they generate what is known as a chiral environment that allows distinctions to be made between how stereoisomers will react.

Optical activity of stereoisomers provides a means for identification between right- handed and left-handed stereoisomer. An instrument known as a polarimeter is capable of measuring extent and direction of rotation of polarized light passed through the stereoisomer. If rotated clockwise, it is considered dextrorotatory; if rotated counterclockwise it is levorotatory. A polarimeter can also determine the optical purity or stereospecificity of the product.

Other methods of determining the stereospecificity include x-ray crystallography and chiral chromatography. Liquid chromatography also provides a method of separating stereoisomers. The complexity of these processes becomes multiplied when more than one chiral center is present in a single compound.

Production of stereospecific compounds is challenging and complex. The only known reliable means of producing a single stereoisomer is based on the utilization of an **enzyme catalyzed process**. This method is known as stereospecific, and of course is the method uniformly utilized by living organisms.[6]

There are multiple methods available for separating racemic mixtures. Most are based on physical characteristics. Derivatization is the most widely available method for separating stereoisomers. Hundreds of reagents have been developed in recent years.[7]

There are many pharmaceuticals in routine use that exemplify the importance of recognition and separation of stereoisomers. Almost everyone has ingested a non-steroidal anti-inflammatory drug (NSAID) at some time for relief of pain from a painful inflammatory process. One of the common drugs in the NSAID category is Naprosyn or Aleve. The chemical names for naproxen and naproxen sodium are (S)–6–methoxy–alpha–methyl–2-

naphthaleneacetic acid and (S)–6–methoxy–alpha–methyl–2-naphthaleneacetic acid sodium salt, respectively. The chemical formulae are $C_{14}H_{14}O_3$ and $C_{14}H_{13}NaO_3$ respectively. The (S) identifies the right-handed stereoisomers which is crucial since the left-handed isomers are highly toxic to the liver.

Another example is an antidepressant drug known as citalopram in which one of the isomers is 170 times more potent than the other. Darvon relieves pain whereas its stereoisomer is a cough suppressant. Ketamine is an anesthetic whereas its stereoisomer has hallucinogenic effects. Can you imagine the chaos caused in an operating room if these two stereoisomers were inadvertently interchanged!

As you have correctly surmised by this point, the Federal Drug Administration (FDA) exercises extensive regulation of the pharmaceutical industry's management of their stereochemistry activities. Their policies are based on the assumption that stereoisomers are "separate drugs" and therefore must be managed accordingly.[8]

**Secondly, what is the statistical probability of random, simple chemistry generating a double helical biochemical molecule, totaling more than three billion base pairs in length encoding a biological encyclopedic instruction manual for building and operating a living organism consisting of approximately 37.2 trillion cells in the average human?**

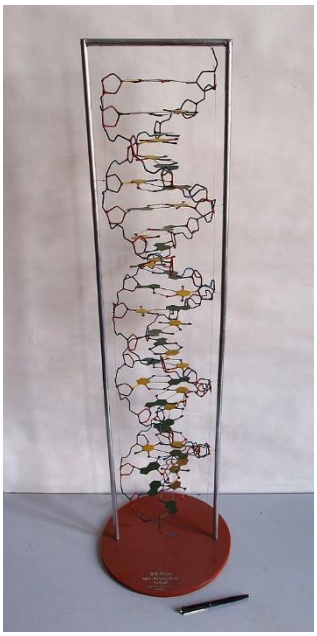


**Figure 3. This picture is of James Watson and Francis Crick being congratulated by Maclyn McCarty for their work on accurately delineating the molecular configuration of DNA. The double helical configuration of the DNA molecule is modeled on the right.**

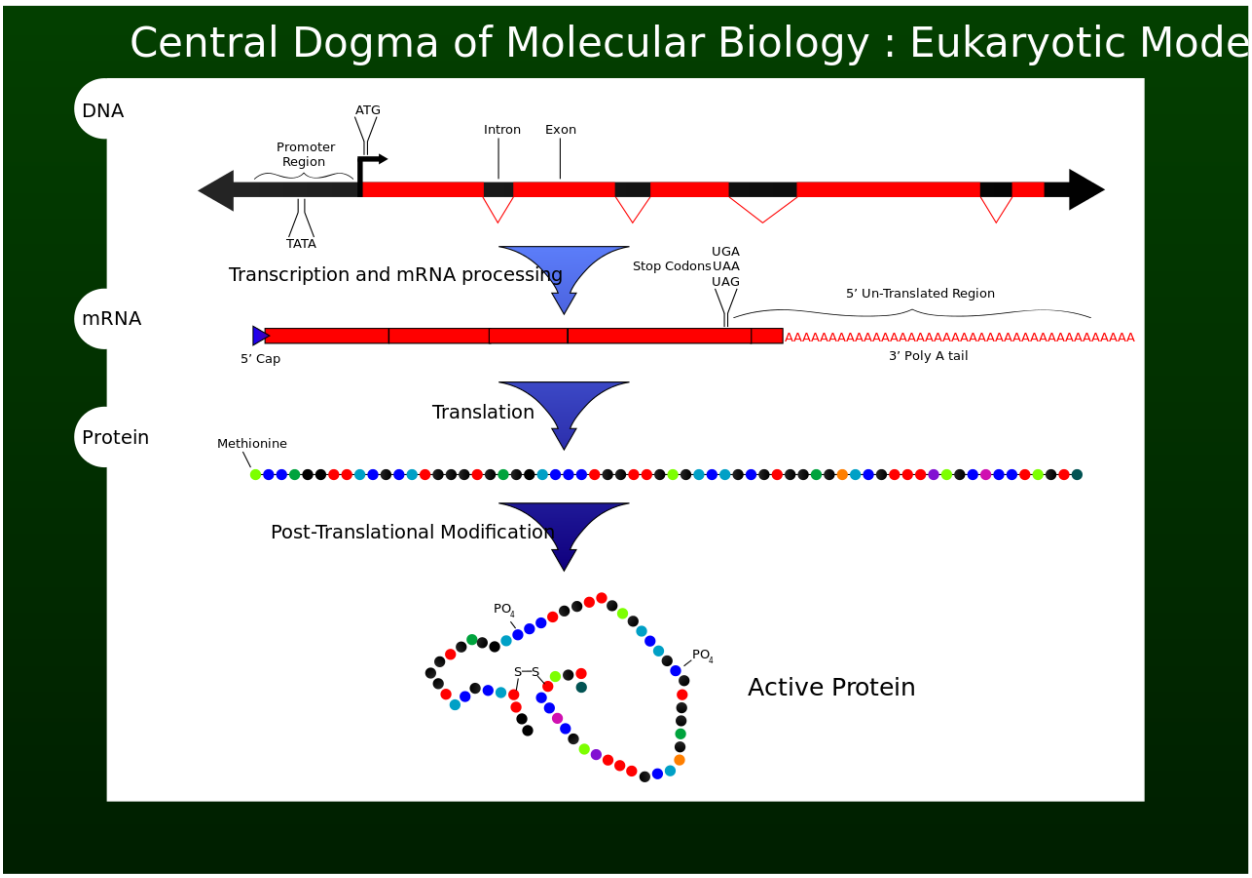
## RNA's Role in the Central Dogma of Molecular Biology

The synthesis of messenger RNA (mRNA) by living organisms is performed by a series of RNA polymerases designated by I, II, III, which are present in the nuclei of all eukaryotes. RNA polymerase IV synthesizes siRNA in plants and RNA polymerase V synthesizes RNAs involved in siRNA–directed heterochromatin formation in plants. These are large complex protein enzymes that are actually nanomachines. We will focus on RNA polymerase II which is responsible for transcribing all protein coding genes and is therefore involved in synthesizing mRNA.

**Figure 4. Schematic Depiction of the Central Dogma of Molecular Biology.**

The top line illustrates a segment of a DNA molecule that is undergoing **transcription** of the gene resulting in a molecule of mRNA. The beginning of the transcription process occurs at the promoter region and continues to the right. Initially the strand is copied in continuity. Subsequently the introns, depicted in black are cut out and the remaining exon portions, depicted in red are spliced together to form the mRNA molecule. This process is labeled mRNA processing. The next step consists of **translation** of the mRNA into a protein molecule that occurs in the ribosome. The long protein molecule polymeric is then folded to form an active protein molecule. In summary the **central dogma of molecular biology** involves **replication of DNA,** followed by **transcription** to form mRNA that is **translated** into protein.

Note that the protein molecule begins with methionine which always occurs according to the **universal genetic code**.



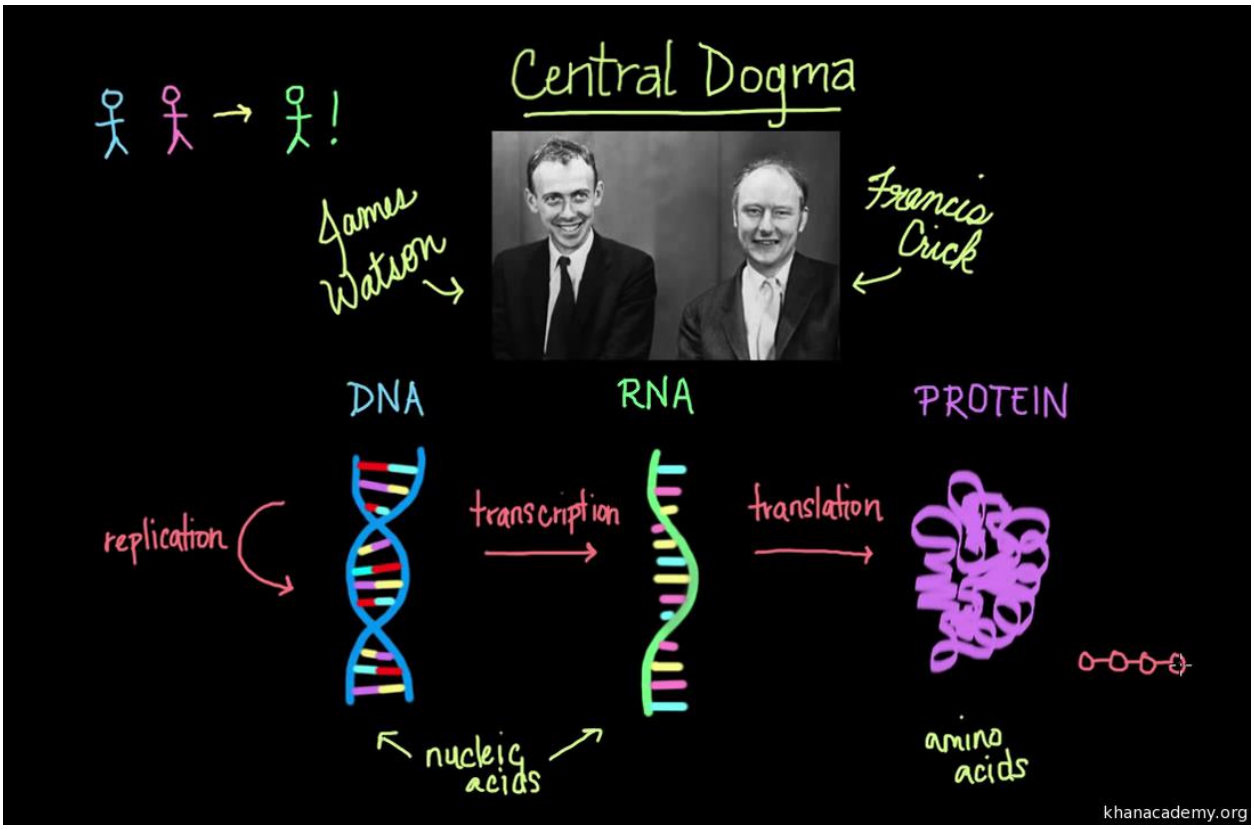


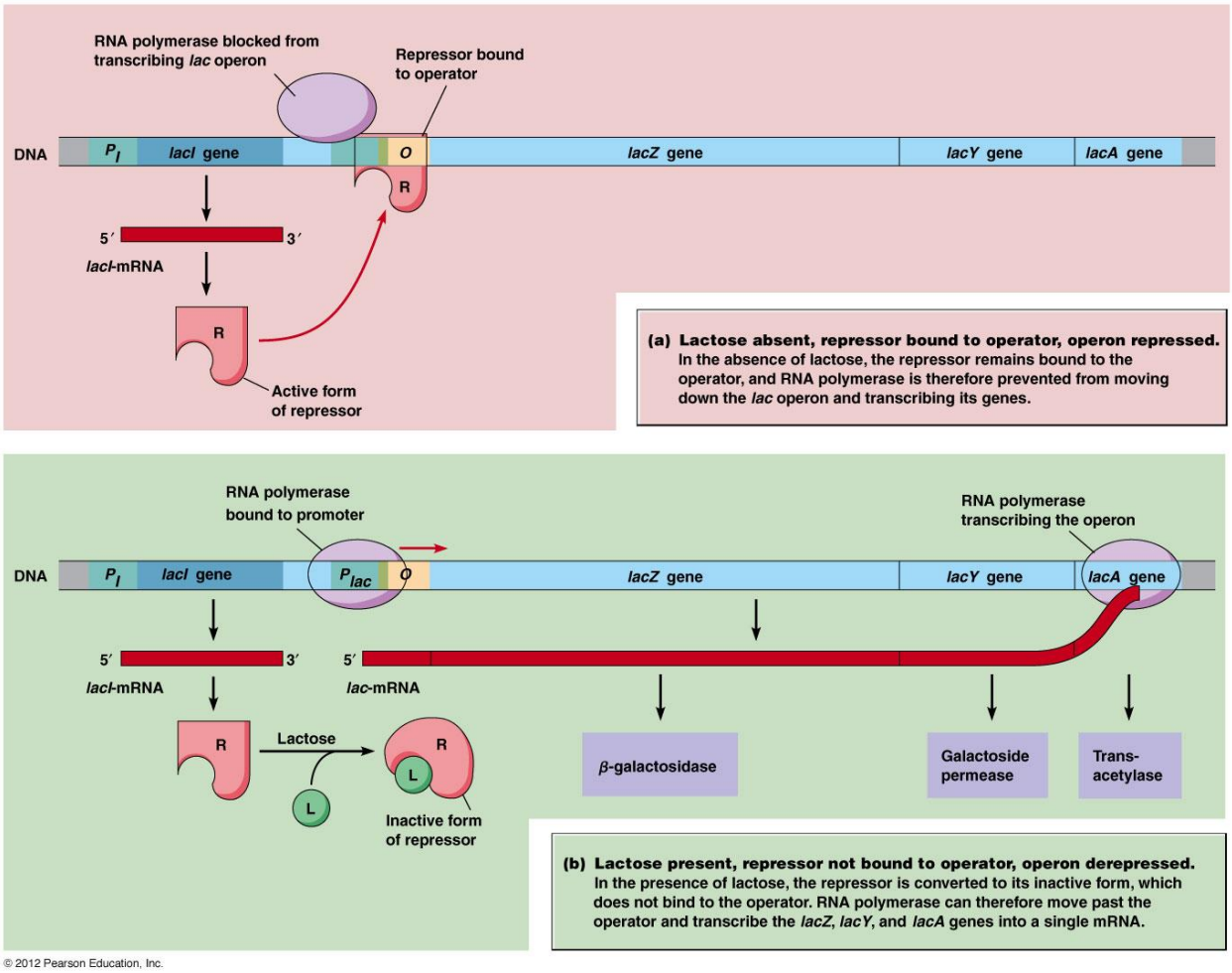

**Figure 5. Photograph of James Watson and Francis Crick, the discoverers of the double helical configuration of DNA and their recognition of the encoded information for building proteins leading to the recognition of the Central Dogma of Molecular Biology.**

In all eukaryotes DNA is located within the nucleus of the cell and in all prokaryotes it lies in the chromatin. Protein synthesis consists of several stages, starting with a signal, known as an activator, arising in the cell, signaling the need for a specific protein. The promotor activates **RNA polymerase II** to function by identifying the specific gene encoding the required protein. The entire mechanism responsible for identifying the exact gene to be transcribed is complicated and only partially understood. A set of rules governing a portion of the cases has been worked out however many other situations are not understood. The phosphorylation of the carboxyl – terminal domain (CTD) has been shown in chromosomes in *Drosophilia* salivary gland preparation to be associated with regions of active RNA transcription sites.

The double helical strands of DNA disassociate at the site of the gene initiating the transcription process of mRNA. A single strand of RNA

representing a precise copy of the sequences of base pairs in the DNA gene segment is generated. The next stage, known as pre-messenger RNA processing, consists of removal of the introns, by cutting them out and splicing the exon portions of the RNA back together, eliminating the introns, of the RNA molecule formerly known as "junk DNA."

**Figure 6. Schematic depiction of Gene Control Involving Lactose**
These slides illustrate one of the early documented cases of gene expression control by nongenetic molecules. It occurs in E. coli in the absence of lactase. In the top slide the gene repressor is active and is firmly bound to the operator (yellow box) on DNA molecule, blocking the progress of RNA

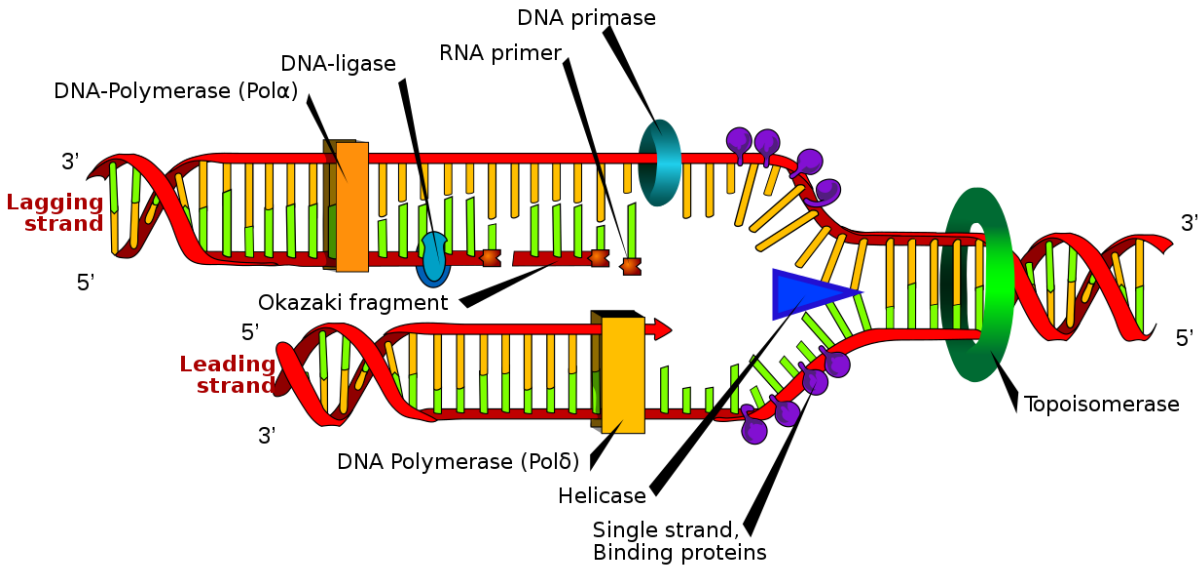




polymerase from proceeding along the DNA strand, thereby preventing the transcription of lac mRNA. This occurs in the absence of lactose It is thought to be a protective mechanism for E. coli to avoid producing lac mRNA for substrate that is not available for metabolism. Glucose is the preferential energy source of E. coli, so in the absence of glucose and the presence of lactose, the gene repressor is turned off by the lactose molecule, allowing

RNA polymerase to move forward producing mRNAs for lac Z, lac Y, and lac A, as depicted in the bottom slide.

**Figure 7. DNA Replication by DNA polymerase**




Before DNA replication can occur specific sites known as **replication origins** must be identified on the DNA molecule. Specific **helicases** (blue wedge) bind to the site and begin the unwinding process of the parental DNA double helix before replication can begin. The DNA polymerase can grow the new strand only in the five 5′ – 3′ direction and can begin duplication of the leading strand without interruption. Since the two strands of the double helix molecule are anti-parallel, replication of the lagging strand is much more complicated and consists of forming short segments in the opposite direction in which an RNA polymerase starts the replication process by forming short strands of RNA primer which become attached to the newly formed replicated DNA strand known as Okazaki fragments. Subsequently the RNA primers are removed from the Okazaki fragments which are subsequently joined together by DNA ligase, reestablishing continuity of the newly synthesized lagging strand again in the 5′ – 3′ direction. The above-described diagram indicates unidirectional replication of DNA however there are multiple studies supporting simultaneous **bidirectional** DNA replication.

The large green colored ring surrounding the double helix on the right of the slide, labeled topoisomerase, provides an important function related to controlling or eliminating left-handed super coiling that may result when the double helix is unraveled during DNA transcription or RNA replication. Since the ends of the double helix are not free to twist, uncoiling of a relatively long segment of DNA causes left-

handed super coiling. The topoisomerase may at times relieve the super coiling by cutting one of the strands and then rejoining them after the normal coiling has been restored.

It should be noted that helicase is a large complex macromolecule. Not shown are multiple copies of heterotrimeric protein RPA and a series of slightly different DNA polymerases also required.

**Deciphering the Universal Genetic code**

There are only four letters in this unique genetic alphabet, corresponding to the four nucleotides: adenine (A), thymine (T), cytosine (C), and guanine (G) that make up the base pairs of the DNA molecule. The RNA molecule is similarly composed with the exception that uracil (U) replaces thymine. These four letters make up the alphabet on which the genetic code is based. The code uses only three letter words based on the sequences of the nucleotides along the DNA strand. With a total of four letters, the number of possible combinations of three letter words is 64, ($4^3$). Each set of three nucleotide sequences is known as a codon; 61 of the codons encode the 20 essential amino acids. The remaining three are stop codes indicating the end of the strand of amino acids to be incorporated into the protein under construction. The number of codes available for each of the 20 amino acids varies between one and six.

Methionine is uniformly located at the beginning of all polypeptide chains of all proteins. This is true of all prokaryotic and eukaryotic cells. The end of the polypeptide chain is indicated by a stop code. The sequence of codons composing the entire polypeptide chain is referred to as a **reading frame**. Thus, each **reading frame** begins with the code for methionine, AUG, and ends with a stop code, one of the following three, UGA, UAA or UAG.

A most remarkable finding is the fact that a given codon, codes the same amino acid in essentially all living organisms including bacteria, yeasts, fungi, animals and plants. As a result, a **Universal Genetic Code** has emerged based on genetic studies across a broad selection of living organisms. The **Universal Genetic Code** is defined as "the instruction manual that all cells use to read the DNA sequence of the gene and build a corresponding protein."[9] There are a few scattered exceptions to this rule that mainly occur in mitochondrial DNA that is thought to have resulted from later evolutionary developments. There is no evidence to support a major overhaul or alteration of the general genetic code since its earliest function.

**"The discovery that virtually all forms of life use DNA to encode their genetic information and also use nearly the identical genetic code, implies that all forms of life descended from a common ancestor based on the storage of information encoded in nucleic acid sequence."[10]**

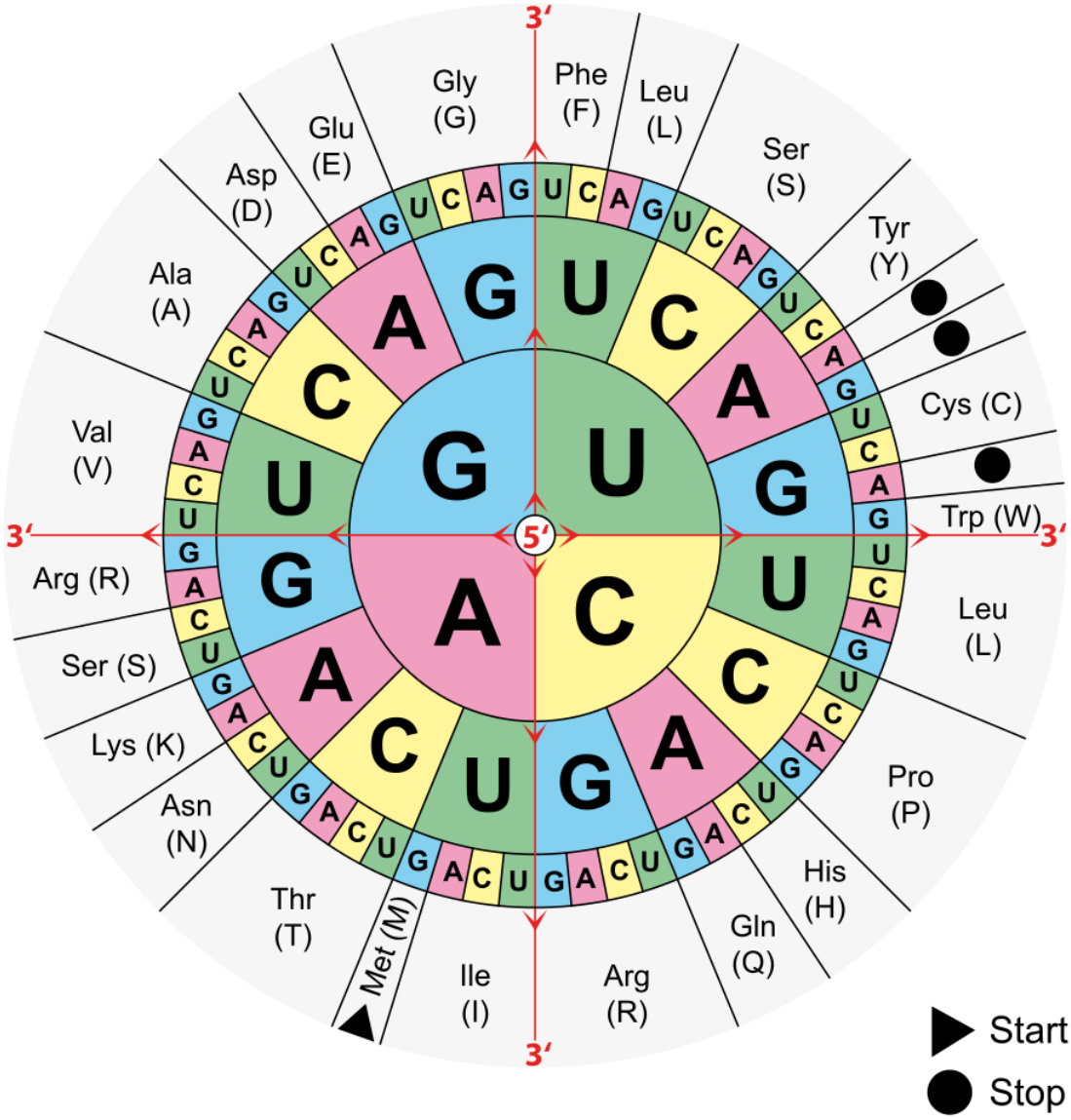



**Figure 8. Universal Genetic Code**

The DNA is not only a most stable molecule as noted earlier but it is also accurately duplicated making it a highly reliable information transfer molecule, ensuring the integrity of inheritance factors of a species from

generation to generation for many millions of years. The error rate of duplication is less than one in $10^9$ nucleotides per generation. It contains all the information required by the fertilized ovum to develop into a full grown human being, estimated at 37.2 trillion cells[11] and the extensive specialized organ systems capable of performing a lifetime of activities including conscious awareness and memory recordings of the whole experience, all accurately encoded in the $3x10^9$ base pairs making up the human genome.

Just as there is constant supervision of the transcription process of DNA in which every nucleotide is carefully examined to ensure that no transcription errors are occurring, there is careful surveillance of the various stages of mRNA synthesis and distribution within the cell. One of the most complex processes in the synthesis of mRNA is the splicing and rejoining of the severed segments. Errors here could give rise to improperly constructed proteins. In all eukaryotic cells there is a mechanism that prevents the transport of defective mRNA molecules leading to their degradation prior to reaching a ribosome.[12]

**"Junk DNA"**

It should be explained that the introns previously incorrectly referred to as "junk DNA" are now known to perform important regulatory functions of the timing, cell type, and the location of cell synthesis particularly during embryonic development. A growing list of genetic diseases associated with mutations of introns is being recognized as more attention and studies are being applied to these regions of the genome. The list includes pre-axial polydactyly (extra fingers or toes), pancreatic agenesis (congenital absence of the pancreas), Pierre – Robin sequence (an often-lethal extensive collection of unrelated congenital malformations), Hirschprung's disease (congenital mega-colon) and a number of types of cancer to list a few. These are known as enhancers that represent dynamic elements of the genome in contrast to the more permanent fixed genes. These are considered to be genome related phenomena included in the emerging field of epigenetics. There may be as many as 20-50 thousand potential gene enhancer elements for each cell type.[13]

**Can Encoded Information appear without an intelligent source?**

The combined information stored in all the DNA molecules composing each individual genome, contains a complete living blueprint for each individual of

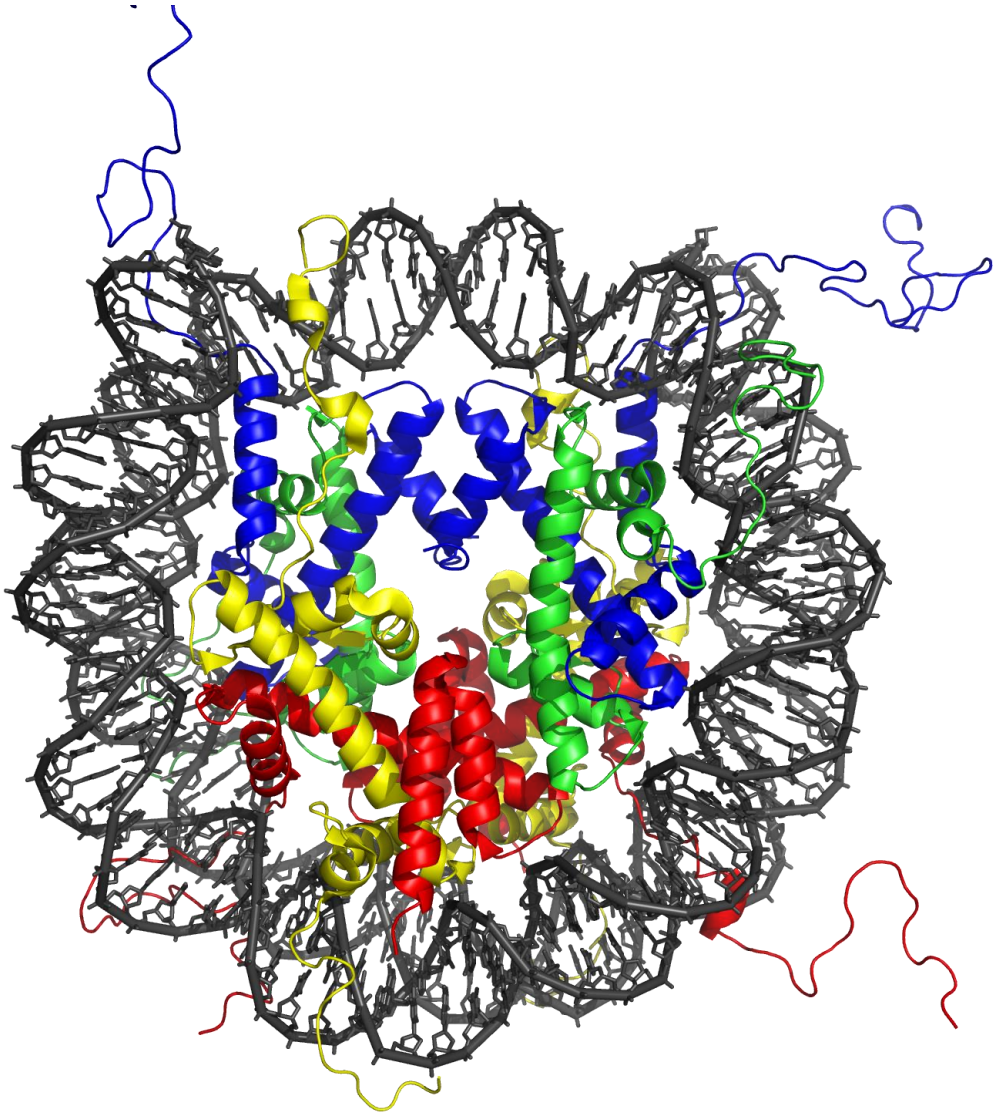
each species for the building and coordination, of the incomprehensible complexity of managing and controlling the innumerable nanomachines operating in every living cell, beginning with the fertilized germ tissue, directing embryonic development, controlling environmental adaptations and finally ending with the death of the organism. Much has been learned but much still remains a mystery. The greatest dilemma facing OOL scientists are embodied in the questions: Where did the information come from and how did it get encoded in DNA?

Review of a current research article, published in *Nature Communication,* December 2018, provides insights into a number of specific aspects of RNA synthesis. The title of the research paper "Structure of Transcribing RNA Polymerase II-Nucleosome Complex," investigates RNA transcription process utilizing DNA strands in chromatin template form. Previous imaging studies have been performed by RNA polymerase II, were done during transcription on linear strands of DNA but not on DNA in form of chromatin templates, the form in which DNA is stored. In chromatin template form, the double helix strands of DNA are wrapped around a core like structure composed of histones, similar to thread wrapped on a spool.

Like so many of the molecular structures we encounter in living organisms, histone molecules are not passive. They are associated with long extensions called histone tails that play an active role in a relatively recently discovered complex process known as **epigenetics**. Epigenetics refers to processes that instruct cells how and when to read the DNA blueprint. They act to control the level of activity of DNA gene expression. The histone tails are able to control the degree of compactness with which the DNA molecule is coiled and thereby affect accessibility to the transcription process which requires an uncoiling of the molecule. These combined structures are known as nucleosomes that when strung together, form the bead like appearance of strands of chromatin.

**Figure 9. High-resolution macromolecule of a strand of DNA wrapped around a nucleosome.** The red yellow green and blue molecules centrally located represent the four pairs of histones complexes combined to form the nucleosome. The compactness of the coiling of strands of DNA makes possible the storage of such immensely molecules to be stored within a single cell nucleus.

The black and gray double helical configuration represents a strand of DNA wrapped relatively tightly around the nucleosome similar to thread wrapped on a spool. The histone tails function to control or modify the level of gene expression based on how tightly the DNA strand is held in position. This represents a biomolecular basis for epigenetics.



**Thirdly, what scientific evidence is there to support a gradual progressive march toward increasing complexity of cells leading to the synthesis of on average 10,000 protein molecules, averaging 400 amino acid molecules in a precise sequence, in which there are 1**

**million exact copies of each protein molecule, all continuously functioning in the average cell?**

Cells are the basic building blocks of all living organisms. All cells are surrounded by plasma cell membranes. The cell membrane is a vitally important living structure in that it provides an interface or boundary between the cytoplasm in which the living functions are performed and the external environment of the cell. It compartmentalizes life's functions.

One of the most ubiquitous features of the plasma membrane is the electrical potential across the membrane in which the electrical charge within the cell is usually negative, ranging from -80 mV to -40 mV, in reference to the external electrical potential. This is a feature of virtually all living cells, including all eukaryotes and prokaryotes. To maintain such an electrical potential, known as the **resting membrane potential**, requires a constant source of energy, a condition, essential for all cell life.

Most cell membranes are bi-layered consisting of two layers of phospholipids in which, when dissolved in water spontaneously form bi-layered spherical cystic structures grossly resembling cell membranes. Many OOL scientists emphasize this spontaneous propensity of phospholipids to form cysts of varying sizes when dissolved in water as a potentially significant factor in prebiotic natural synthesis of cells. The phosphoglycerides are the most common phospholipids in cell membranes. They consist of two very different segments in terms of their reactive potential with water versus fat molecules, in that the head portions of the molecule are positioned away from one another and are strongly **hydrophilic** (water loving) whereas the tail portions of the molecule consisting of short hydrocarbons chains are positioned toward one another, forming a strongly **hydrophobic** (water avoiding) layer in the central portion of the membrane.

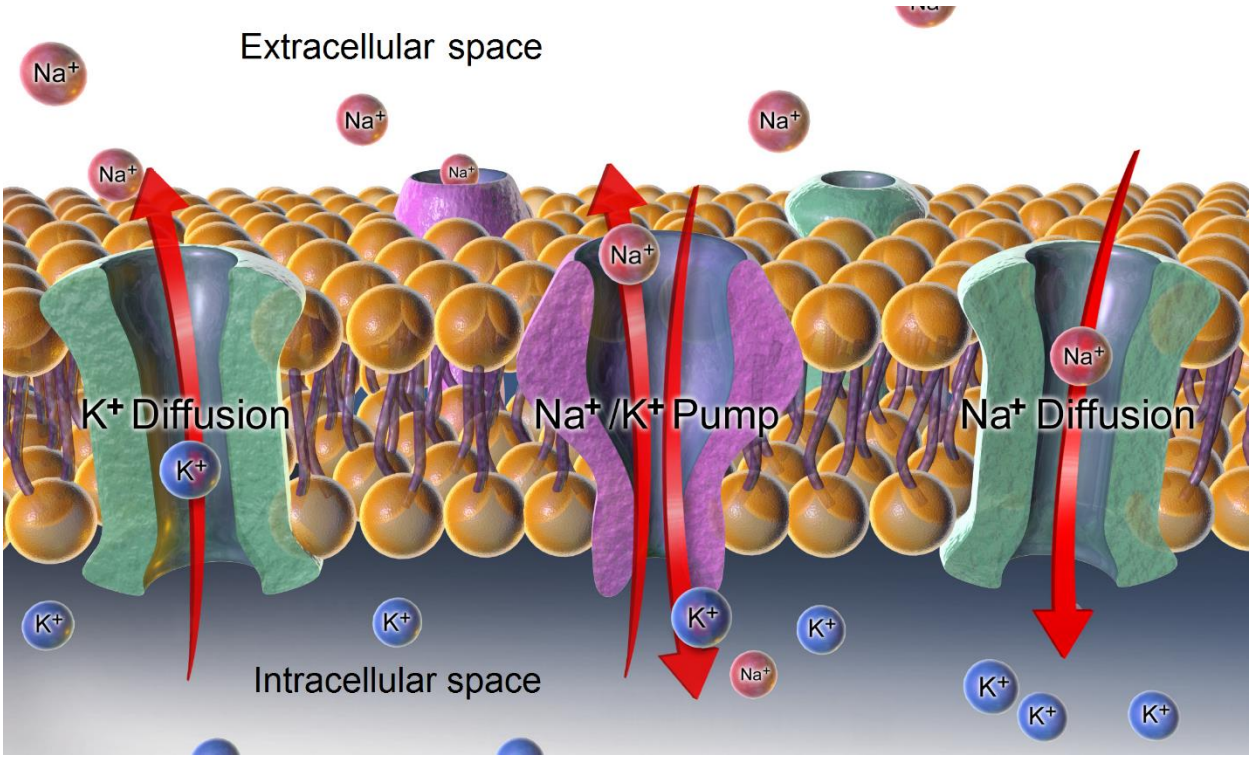
The bilayer cell membranes consisting of phosphoglycerides are far from homogeneous. The bilayers, one forming exoplasmic face, the other representing the cytosolic surface, vary significantly in their specific phospholipid components. Many unique features are probably a result of their origin of synthesis, that is whether they are produced by the endoplasmic reticulum, Golgi Apparatus, or another source. There are many other variations related to shape such as cylindrical configuration producing a flat surface or cone-shaped producing a curved surface. The cell

membranes are highly dynamic structures with constant modifications, substitutions, and alterations.

But it is the proteins in the membrane which provide its most unique and highly varied functions necessary to support life. These include proteins that provide passage ways, or ion pores, through the cell wall allowing specific ions to pass either in or out or both directions; there are lipid anchor proteins tethered to one leaflet by a covalently attached hydrocarbon chain; there are peripheral proteins associate with the membrane primarily by specific noncovalent interactions with integral proteins; there are membrane lipid commands in the plasma membrane responsible for contact with the cytoskeleton and integral membrane proteins, of various types providing active transport of a wide range of proteins and nutrients essential for life.

The maintenance and control of the negative transmembrane electrical potential across the cell membrane required for all living cells, is a complex process requiring careful balancing of transmembrane passages of all charged moieties exiting and entering the cell.

**Figure 10. Sodium – Potassium Transmembrane Pump**



The sodium ($Na^{+)}$/potassium ($K^{+)}$ pump is one of the most important basic nanomachines in all living organisms. It is a large complex transmembrane protein, extending across the cell membrane capable of active transport. It can pump three sodium ions out and two potassium ions into the cell with each cycle, requiring one molecule of ATP. Thus, the intracellular fluid becomes one charge more negative relative to the extracellular fluid. In addition, the positive concentration gradient of potassium ions across the cell membrane is increased well as the negative concentration gradient of sodium ions. Other significant functions of the sodium potassium pump include maintenance and control of osmolality by controlling cell volume, import of glucose and amino acids and other nutrients into the cell by use of the sodium gradient, it contributes to the absorption of glucose and certain other nutrients from the upper intestinal track and plays a role as a signal transducer as extracellular ouabain-binding into the cell through regulation of protein tyrosine phosphorylation.

Since the sodium – potassium transmembrane pump requires energy, a second large macromolecular nanomachine capable of generating ATP is required to maintain the transmembrane potential. ATP synthase is the commonness source of ATP throughout the animal kingdom. As illustrated below is a large macro molecular nanomachine consisting of multiple moving parts and large protein molecules forming a large complex.

ATP Synthase enzymes are the primary fuel source for almost all living organisms. These are complicated nanomachines varying in size and complexity but in all cases they are highly complex. They are built by ribosomes, the basic protein factories in living cells. This brings us to the fundamental question related to the concept of irreducible complexity that Darwin himself posed. In *The Origin of Species* (1859), he wrote, "If it could be demonstrated that any complex organ existed, which could not possibly have been formed by numerous, successive, slight modifications, my theory would absolutely break down. But I can find out no such case."[14]

We are faced with the dilemma that Darwin anticipated. That is is the improbability of large "irreducibly complex" biological structures coming into existence by a long series of gradual advances based on random mutations. As Darwin admitted, he had no way of knowing of the complexities of living organisms that are now being discovered at an unprecedented rate as a result of our ability to observe life at the molecular level.

## ATP Synthase

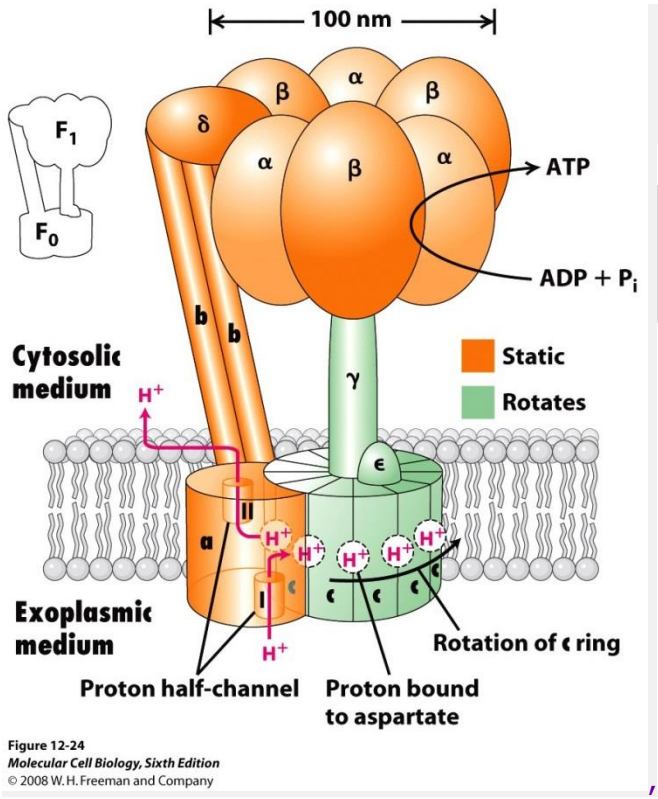



**Figure 11. Courtesy of Protein Data Bank. ATP Synthase: schematic drawing**

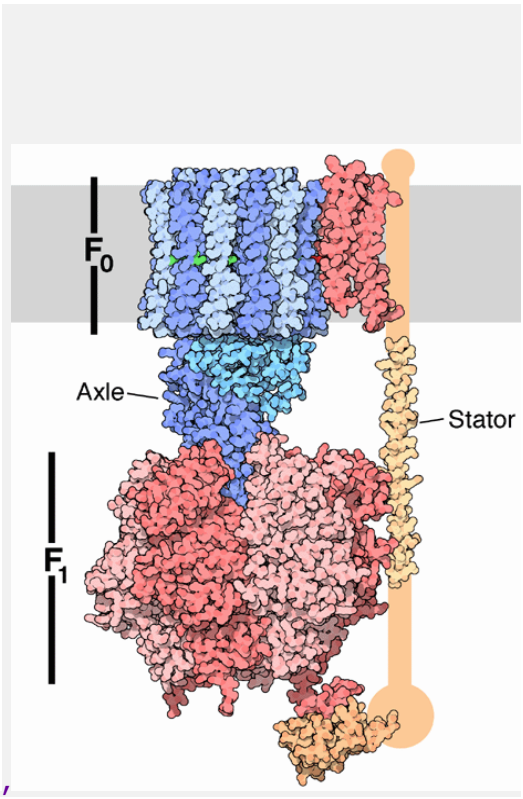



**Figure 12. Courtesy of Protein Bank ATP Synthase: Macromolicule**

(Note: the orientation of figure 12 is rotated 180 degrees in reference figure to 11.)

ATP synthase is a widely distributed macromolecular nano-machine located in mitochondria. (See figures 2 and 3.) It is the primary generator of ATP throughout the entire animal kingdom. It is a complex multi-protein with an overall cylindrical configuration. It has a column like protein structure rigidly attached in a vertical manner so that when the cylindrical structure, acting as an armature rotates, the column turns with it like a driveshaft. The cylindrical shaped portion is positioned in the plane of the mitochondrial membrane. It consists of ten to fourteen, pie shaped identical protein molecules (c's), there are twelve in humans, that fit together to form a cylindrical shaped macromolecule. Each of the pie shaped protein structures is labeled c. Its rotation is powered by protons flowing, one at a time, along a pathway consisting of two half channels, leading from the exoplasmic medium through the mitochondrial wall and emptying into the interior

cytosolic medium. Since one proton is required to rotate the armature by one c unit, in humans, twelve protons are required to produce one complete rotation.

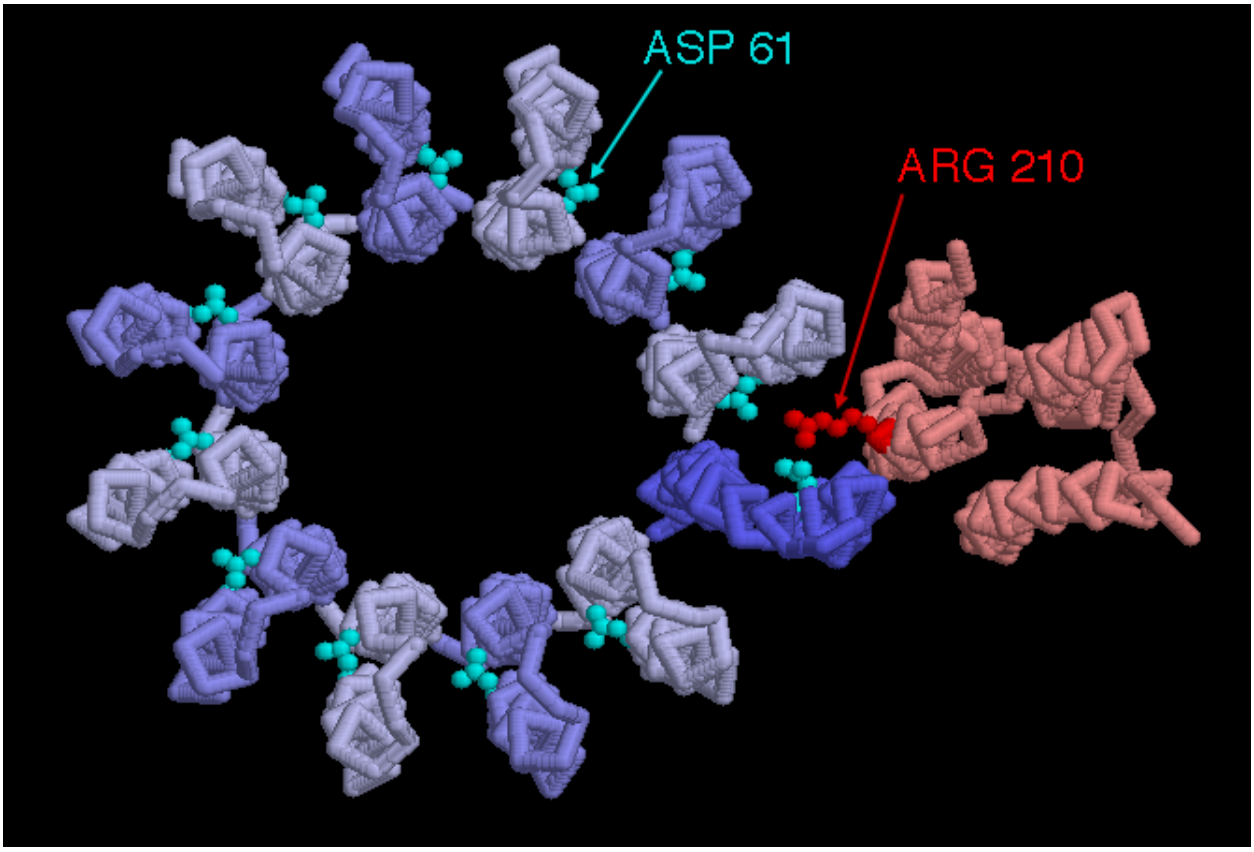



**Figure 13. Courtesy of Protein Data Bank**

ATP Synthase, FO structure, top view, showing the 210 position on the arginine amino acid and 61 position on aspartic amino acid. These represent the binding sites.

As a proton flows through the first half channel, at approximately midway through the wall, it interacts with Asp (aspartic acid) 61 on its un-protonated binding site, resulting in balancing a negative charge on this same side chain of the amino acid. It is also partially balanced by a positive charge on nearby Arg (arginine) 210. "The proton fills the empty proton-binding site and simultaneously displaces the Arg 210 side chain, which swings over to the filled proton binding site on the adjacent c subunit. As a consequence the proton bound at that adjacent site is displaced. The displaced adjacent proton moves through half channel II and is released into the cytosolic space leaving an empty proton-binding site on Asp 61. Counterclockwise rotation

of the entire c ring moves the "empty" c subunit over half channel 1." Rotation is caused by thermal/Brownian motion, and this is all powered by "proton – motive force" across the membrane that drives the flow of protons through the membrane, from the exoplasmic to the cytosolic medium.

As mentioned earlier, there is a static (s) component which is actually larger in over-all size than the rotating component. It measures slightly more than 100 nm in diameter. It is firmly anchored in the mitochondrial membrane. There are two large paired linear structures with an additional intervening protein macromolecule that support a large donut shaped structure, consisting of three pairs of alternating macromolecules (F1). These sit on top the rotor shaft associated with m, described earlier, much like the head of a mushroom sits on its stalk.

Each pair of the macromolecules making up the mushroom head-like structure, occupy 120° of the 360$^{o}$ ring. There is an asymmetrical molecule attached firmly to the upper end of the rotating shaft of m. This structure functions as a cam. As the cam rotates each 120° segment of rotation it makes intimate contact with the binding sites in each of the three paired macromolecules where it causes a change in the conformational state in each. (There is an actual change in the shape of the protein structures causing a mechanical shift at each of the binding sites.) As it rotates, it cycles through each of three stages at the binding sites. The first stage is the releasing stage of a molecule of ATP; the second stage is weakly attracting a molecule of ADP + $P_i$; and the third stage is strongly attracting the ADP + $P_i$ resulting in a firmly bound molecule of ATP, ready for release into the cytosolic medium.

The rate of rotation has been experimentally measured at approximately 134 revolutions per second. The rate of generation of ATP molecules has been experimentally measured at about 400 ATP molecules per second. Since three molecules of ATP are produced and released into the cytosolic medium with each rotation, these experimental values are in excellent agreement.

Well-recognized feedback systems controlling the rate of ATP synthesis are recognized such as the concentration of ADP. There is also a coupling of oxidation of NADH and $FADH_2$ to the synthesis of ATP, so if the resulting proton–motive force is not dissipated during the synthesis of ATP, the transmembrane gradient resistance will increase and eventually block further reaction.

In summary, ATP synthase is a large macromolecular nano-machine that occupies one of the most critical positions in the bacteria, animal and plant kingdoms. It is an essential component for almost all living cells. It generates the fuel required to power all of the major physiological functions of living animals.

It consists of twenty-five distinct macromolecules in which five are singular, four are paired and one consists of twelve (in humans) identical copies arranged in a cylindrical shaped complex protein acting like an armature of an electric motor that rotates at an experimentally determined rate of 134 revolutions per second. (8040 revolutions per minute). It is uniquely situated in the wall of mitochondria and provides a pathway for protons to flow across the membrane, traversing two, one-half, uniquely arranged channels, driven by the proton – motive force. It is estimated that in humans, the amount of ATP produced and utilized each day is approximately equal to one's total body weight. Without ATP all animal life would immediately cease.

**Eukaryotic vs Prokaryotic Cells**

Living organisms are generally grouped into two classifications, Eukaryote and Prokaryotes. Eukaryotes compose the vast majority of living organisms including yeasts and all multicellular organisms, whereas prokaryotes are primarily composed of bacteria and archaea which are almost completely devoid of organelles; they do not have defined nuclei and are much smaller than eukaryotes, approximately one 1000th their size. The energy systems of archaea are surprisingly diverse given their otherwise primitive characteristics. They are able to oxidize $H_2$, $H_2S$ and $Fe^{2+}$, to reduce sulfate to sulfide and $CO_2$ to methane as well as cause nitrogen fixation. More differences are being found at the molecular level as our understanding at this level deepens.[15]

# Prokaryotic vs Eukaryotic Cells

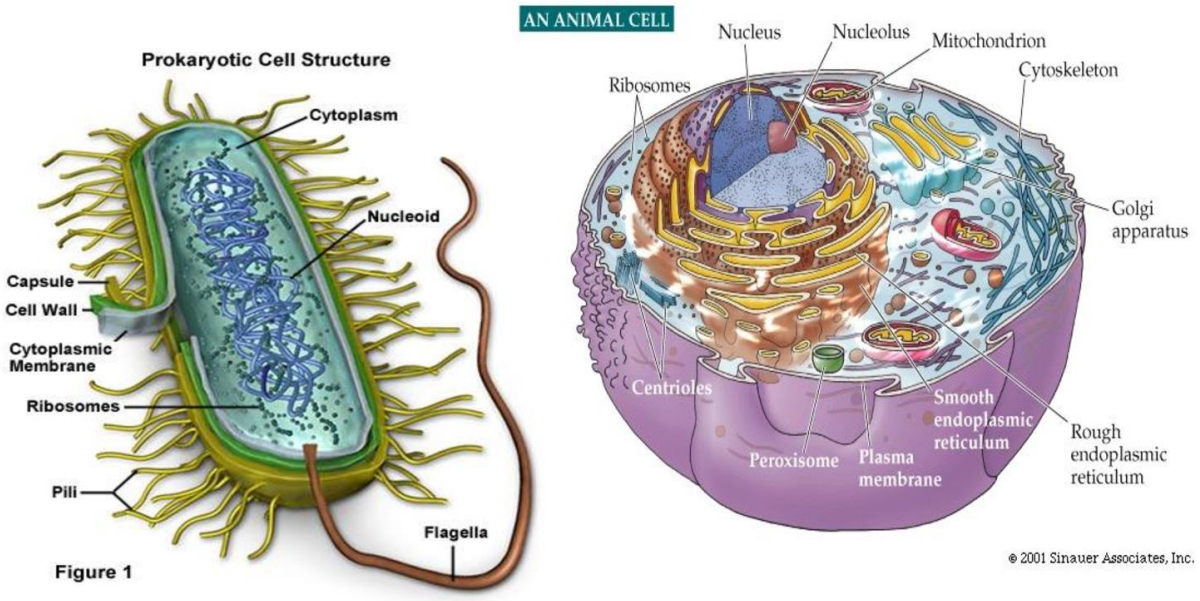



**Figure 14.** Diagrammatic Comparison of a Prokaryote (E. coli) and a Eukaryote Cell. In addition to the marked difference in size, the eukaryote being approximately 1000 times the prokaryote, the DNA in a eukaryote is confined within a nuclear membrane whereas it is loosely distributed as chromatin fibers in the prokaryote. Other major differences are the presence of organelles such as mitochondria, cytoskeleton, Golgi apparatus, rough endoplasmic reticulum, smooth endoplasmic reticulum, peroxisome, and centrioles in eukaryotes which are completely absent in prokaryotes.

*E. coli,* have been extensively studied because of their simplicity, availability and ease with which they reproduce. Their complete genome has been deciphered. Though one might have expected to find far simpler proteins and less complex intermolecular interactions, then those observed in eukaryotes, that has not proven to be the case. Electron microscopy has revealed a relatively large collection of DNA molecules in an area termed the nucleoid, generally located at the center of the organism; there are also many small ribosomes in the cytosol. Complex interactive processes

between DNA, mRNA, and protein synthesis occur. E. coli have multiple flagella providing impressive mobility. They can react to their environment by moving toward nutrient sources, as well as slowing their metabolism rate when nutrients are scarce. They can reproduce rapidly, undergoing cell division every 20 min. under ideal circumstances.

An example of what we can expect with further study of prokaryotes, is the recent documentation of aquaporin in E. coli. It is highly specific and displays a rapid rate of water flow through its channel. It is expected to provide a useful model for further study of aquaporins. They are large complex macromolecular proteins located in cell membranes and in general are responsible for controlling the flow of water and glycerol in and out of cells.[16]

Even though prokaryotes display relatively few subcellular structures, there appears to be significant subcellular organization on a molecular level. Microbiologists, Lucy Shapiro and Richard Losick, stated, "The use of immunogold electron microscopy and fluorescence microscopy to study the sub-cellular organization of bacterial cells has revealed a surprising extent of protein compartmentalization and localization." They go on to describe such examples as DNA polymerase, cell division proteins, and bacterial cytoskeleton.[17]

**Eukaryotes** populate the bodies of the entire animal kingdom and represent our general concept of a typical cell. They display extensive intracellular compartmentalization, a nucleus surrounded by a galaxy of highly sophisticated organelles performing specific functions. Their energy systems are well-defined and highly specialized. Chloroplasts in plants serve as solar panels, transforming the energy of sunlight into chemical energy in the form of sugars, starches and ATP, whereas in animals, mitochondria transform the stored chemical energy in the ingested sugars and starches, as well as fatty acids, into adenosine triphosphate (ATP). Energy can also be extracted from excess ingested proteins and amino acids in the diet

Franklin M. Harold , professor emeritus of biochemistry at Colorado State University, whose professional career spanned 40 years of research focused on microorganisms, stated in his recent book The Way of the Cell, "Biochemists insist, rightly, that when one takes cells apart one finds nothing but molecules: no forces unique to life, no cosmic plan, only molecules whose writhings and couplings underlie and explain all that the cell does.

Thus Max Perutz, reflecting on the mechanisms that allow E. coli to detect and swim towards a source of nutrients, found nothing that could not be "reduced to chemistry" (3). I share the commitment to a material conception of life, but that makes it doubly necessary to remember that before the cells were taken apart – as long, in deed, as they were alive – they displayed capacities that go beyond chemistry. Homeostasis, purposeful behavior, reproduction, morphogenesis, and descent with modification are not part of the vocabulary of chemistry but point to higher levels of order. Even as a catalog of small parts approaches completion, the transition from molecular chemistry to the supramolecular order of cell emerges as a prodigious challenge to the imagination. Make no mistake about it: here we touch, if not the very secret of life, at least an essential stratum of that many layered mystery. For if life is to be convincingly explained in terms of matter and energy, organization is all that stands between a soup of chemicals and a living cell."[18]

If one could imagine that somehow on early Earth a primordial soup existed containing all the building blocks that make up a living cell, that is, all the proteins, all the sugars, all the fats, all the bases all the acids, and all the different dissolved ions in appropriate concentrations required to form a living cell, and continue to imagine that all of these components were produced by prebiotic chemistry, even though at present all the research performed thus far on this problem, including “The RNA World”, has not come anywhere close to achieving such results, there still remains the colossal problem of how all of these components could become organized into all the interactive processes required for life. For example, how could one possibly imagine all of the nanomachines required in a living cell to be instantly turned on and immediately begin performing their complex robotic activities in a synchronized manner. Because of its overwhelming complexity the origin of life remains a profound mystery.

## Stability Coexistence with Constant Molecular Synthesis, Disassembly, Recycling and Rearrangement Characterizes Life

Atoms and molecules are the building materials from which all living organisms are made. Life is dependent on thousands of chemical reactions that occur continuously and simultaneously over an entire lifetime. They are exquisitely coordinated, constantly modified, and controlled by the central nervous system, continuously responsive to feedback from internal stimuli,

adjust to external environmental challenges, are directed by thousands of genetic instructions, and greatly influenced by carefully balanced hormonal influences. All of these factors controlling and directing living creatures are based on chemical interactions within the living organism. How the limited number of different elements that compose the many trillions of molecules that carry out these incredibly complex processes in all living organisms can achieve such diverse functions and at the same time maintain the stability and functional predictability so essential to life, remains an unsolved mystery.

The complex processes of life are almost exclusively performed by proteins. Proteins consists of chains of amino acids varying in from a few dozen to thousands in length, known as polymers. There are 20 basic amino acids in humans, nine of which are considered essential; an essential amino acid is one that the human body is unable to synthesize from other protein sources and therefore must be supplied in the diet on a regular daily basis. Our bodies are capable of synthesizing the remaining 11 basic amino acids from ingested proteins. It is important to obtain an adequate balance of the nine essential amino acids on a daily basis. Protein synthesis is dependent on an adequate supply of all of the specific amino acids required. If there are insufficient quantities of one or more of the nine essential amino acids, protein synthesis will be delayed accordingly. A careful analysis of the living proteins indicates more than 100 different amino acids present as a result of modifications of the 20 basic amino acids by phosphorylation, glycosylation, hydroxylation, methylation, carboxylation, and acetylation.

An analysis of the elements required for life reveals a surprisingly narrow selection. Water is by far the most prevalent compound in living organisms composing about 70% of body weight. As a result, hydrogen is by far the most prevalent element, responsible for approximately 50% of all the atoms in living organisms with oxygen the second most common element. Other common elements in descending order of frequency are carbon, nitrogen, phosphorus and sulfur. Additional elements occasionally encountered include calcium, potassium, iron, zinc, magnesium, manganese, fluorine, and iodine. We are all familiar with iron deficiency anemia that may result from chronic blood loss in which iron in the lost hemoglobin cannot be recycled. Another elemental deficiency of the past, occurred in the “goiter belt”, due to dietary deficiency of iodine, resulting from unusually low levels of iodine in the soil

and water was widely recognized in the 19th century. It has since been corrected primarily through the iodization of salt.

Normally our red blood cells are completely recycled every 60 to 90 days in which red blood cells undergo apoptosis and the iron and hemoglobin are completely disassembled, recycled, and re-synthesized. Replacement of red blood cells occurs in the red bone marrow. The recycling of red blood cells was the first tissue to be recognized that was recycled and replaced on a regular schedule. Since, it has been found that essentially all body tissues are replaced on a regular schedule. Exceptions are tissues composing the lens of the eye and neurons making up the central and peripheral nervous system.

The epithelial cells lining of the entire small bowel, that are responsible for absorbing the digested nutrients and transporting them into the bloodstream, are regularly recycled. In fact these absorptive enterocytes display the most rapidly known self-renewal rate of any adult mammalian tissue.[19] The stem cells from which the replacement cells arise, are located in the crypts at the base of the microvilli that are microscopic fingerlike projections lining the inner surface of the small bowel, thereby markedly increasing its absorptive surface area. Starting from the crypts they gradually migrate up to the tips of the microvilli where they undergo apoptosis, allowing space for the newly arriving replacement endothelial cells. The entire cycle requires five days on average. It would appear that maintenance of the highest level of efficiency in absorption of necessary nutrients is of utmost importance for living organisms.

It should be noted that the absorptive process of nutrients in the small bowel is a highly controlled, carefully regulated process including an active enzyme mediated absorption of particular nutrients such as glucose by active transport. This first active transport stage is mediated by an enzyme known as two-$Na^+$/glucose symporter, in which two sodium ions are exchanged for each molecule of glucose absorbed from the bowel lumen. The active transport is driven by an electron potential generated across the cell membrane by potassium ions leaving the cell and by active sodium ion transport into the bloodstream by ATPase. A second phase is accomplished by HansGLUT2 which transport glucose in a downhill concentration gradient into the bloodstream. This process occurs in two stages and is known as *transcellular transport.* The same two stage transport system is also

responsible for transporting amino acids from the intestinal lumen across the cell membranes against uphill concentration gradient and then into the bloodstream which is a downhill concentration.[20]

## Apoptosis

Apoptosis is a well-recognized process defined as programmed cell death. In general it is controlled and mediated genetically in which specific genes are responsible for the synthesis of specific proteins that initiate, control, and regulate the apoptosis process including the recycling, reabsorption, and removal of the products of cell death. Apoptosis makes possible the regulated routine replacement of various tissues noted above. It is of interest that the reason neurons do not participate in this process is due to neurotrophins that specifically block the apoptosis process in neurons.

Another constant turnover that is more difficult to accurately assess is the regular exchange at the elemental level. We are all well aware of the regular exchange that occurs with the water composing our body fluids. A most interesting example of carefully controlled concentration and turnover of a dissolved metallic ion is calcium, with conformational changes induced by calcium binding to calmodlin.[21]

## Mathematical Probability of Evolution Occurring Solely by Natural Means

On April 25 and 26, 1962 a group of prominent mathematicians, biologists, and medical scientists. By all accounts they were a truly distinguished group of evolutionary scientists.

The chairman, Sir Peter Medawar of the national Institute for medical research in London, England, stated the reasons why they had gathered:

"… [T]he immediate cause of this conference is a pretty widespread sense of dissatisfaction about what has come to be thought of as the accepted evolutionary theory in the English-speaking world, the so-called neo-Darwinian theory… These objections to current neo-Darwinian theory are *very widely held among biologists generally;* and we must on no account, I think, make light of them. The very fact that we are having this conference is evidence that we are not making light of them."[22]

Dr. Murray Eden, Prof. of Electrical Engineering at MIT, clearly expressed his dissatisfaction with the role assigned to random changes in the genetic code by Neo-Darwinists. In his symposium paper, he stated "It is our contention that if 'random' [chance] is given a serious and crucial interpretation from a probabilistic point of view, the randomness postulate is *highly implausible* and that an adequate scientific theory of evolution must await the discovery and elucidation of new natural laws, physical, chemical and biological."[23]

Dr. Edens pessimistic assessment of the likelihood of random mutations providing an adequate supply of new proteins, complex new organ systems, and completely new body forms, required to advance evolution was based on the work of Marcel P. Schutzenberger at the University of Paris, France, who calculated the probability of evolution based mutation and natural selection to be essentially zero, ($<10^{-1000}$.) Although this mathematical expression is admittedly completely beyond human comprehension, many other scientists at the time were in agreement with his conclusion.

A major reason that neo-Darwinists did not take these mathematical considerations seriously at the time of the Wistar Institute conference of such a distinguished group of evolutionary scientists in 1962 is that at that time it was felt by many that protein molecular function probably overlapped and their function was not nearly as specifically dependent on the exact sequence and configuration of the amino acids as we now know to be the case.

**The Combinatorial Problem**

The combinatorial problem refers to the probability of reproducing an exact copy of a large collection of different items recurring in a specified sequence or relationship. This is of great significance in the synthesis of large complex molecules consisting of long chains of specific amino acids that to be functional must follow a precise sequence in the polymer.

Recent work by Douglas Axe has greatly clarified our understanding of this problem. He has found that for a protein containing 150 amino acids, (which is actually much smaller than the average of approximately 400 amino acids) there are $10^{77}$ probabilities that it will not fold into the configuration that will provide a correctly functioning stable three-dimensional protein molecule. In other words for every correctly functioning stable three-

dimensional protein molecules consisting of 150 amino acids, the probability of one occurring spontaneously by random selection is one in $10^{77}$.[24]

Is it possible to comprehend a number of this magnitude? (For many thousands of years primitive man was unable to count beyond three.) Most people today are reasonably comfortable thinking in terms of a few thousand and for some, even a few million is vaguely comprehensible, but few if any really perceive a billion or more. A billion is $10^9$ and similarly, a trillion is $10^{12}$, which most people would admit are beyond their comprehension.

An approach that may provide perspective on this problem though not really contribute to an appreciation or comprehension of the size of the numbers themselves is to consider relevant facts with probabilities in the numerical range we are considering. For example, it is estimated that there are approximately $10^{65}$ atoms in The Milky Way Galaxy which leaves most of us with the impression of an inconceivably gigantic number. A fact of much greater relevance however is the recognition that in the entire 3½ billion year history of the planet during which life is thought by some scientists to have been present, (based on highly controversial and extremely limited evidence during the four first 3 billion years), it is estimated that the total number of individual organisms that have ever lived is in the range of $10^{40}$. Because these are exponential numbers, their difference in magnitude remains incomprehensibly large Although the exponents, 77 and 40, are not greatly different, the one a little bit less than double the other, being exponents actually indicates that for each numerical increase in the exponent actually indicates a tenfold increase in the size of the number. As a result, $10^{77}$ is 10 trillion, trillion, trillion times larger than $10^{40}$! From a purely statistical point of view, the probability of a functional average size protein spontaneously evolving even if every organism that ever lived participated, is extremely improbable.

**Turnover Rates of Atoms in Living Organisms**

In the 1930s as artificially produced isotopes made in an atomic reactor or pile became more readily available, a series of studies at Oak Ridge Atomic Research Center by Paul Aebersold, revealed that about 98% of all atoms in the human body are replaced every year. Based on longer-term studies experts have estimated that 100% atomic turnover occurs in humans every five years. His studies were based on administering radioisotopes for medical reasons and then carefully tracking their distribution and subsequent

elimination over time. After administering sodium 24 he found that in a week or two one half of the sodium atoms in the body were replaced. Using phosphorus 32 a few weeks were required for one half of the body phosphorus to be replaced and with radioactive carbon it required a month to two months to be replaced. The most rapid turnover was noted with water which forms about 70% of body weight; one half of total body water turned over in eight days.[25]

As American computational neuroscientist Terrence Sejnowski states in his 2007 query about the memory mechanism: "I have been puzzled by my ability to remember my childhood even though most of the molecules in my body today are not the same ones I had as a child—in particular the molecules that make up my brain are constantly being replaced with newly minted molecules—despite this **molecular turnover**, I have detailed memories of places where I lived fifty years ago."[26]

In the human molecules, the turnover rate of atoms in the body is 98%, meaning that every year, the typical person acquires nearly a complete set of all the 26 different elements that comprise the average human body.

Paul Aebersold , in an address to teachers in 1949 stated interestingly, "Although next year you will be almost a completely new batch of atoms, you won't be a new person. Your supercolossal traffic of atoms does not take place just by chance. It is very carefully regulated and controlled. In general, the atoms do not get very far off the right road, and there are no traffic jams. Next year we will appear much the same as we do now. Even though most of the atoms in our brain will have been replaced by other atoms, we will still go on remembering things that happened a long time ago. Also our emotions, reasoning, personality and individuality go on much the same. Physically we may be a new batch of atoms but unfortunately, perhaps, we are an old batch of emotions, ideas and reactions."[27]

**Number of Protein Molecules in an Average Cell**

Now that we have considered the rate of turnover of atoms that make up the proteins of the human body, we should attempt to determine the number of protein molecules contained in an average human cell. A typical protein molecule is estimated to contain approximately 400 amino acids with a range of 100 to 1000. The volume of an average cell, such as a liver cell, is cuboidal shape measuring 15 μm on a side with a calculated volume of

$3.4\times10^{-9}$ $cm^{3.}$ The average density of soft tissue being slightly heavier than water is in the range of 1.03 g/mL based on the above volume the average cell weighs approximately $3.5\times10^{-9}$ g. The average percentage of the cell weight due to protein is approximately 20% resulting in the average of $7\times10^{-10}$ g of protein per cell. The average molecular weight of protein molecules is 52,700 g/mole. Using Avogadro's number ($6.02\times10^{23}$) the number of protein molecules per liver cell is approximately $7.9\times10^{9}$, or approximately 10 billion. A liver cell contains approximately 10,000 different proteins indicating on average there are 1 million of each of these different proteins simultaneously functioning within the cell.

How each of the estimated 37.2 trillion cells in the average human body maintains an appropriate number of each protein molecule implies highly complex control system at the cellular level to maintain homeostasis throughout a human lifetime.[28]

Our ability to accurately assess the average age of human cells has been greatly expedited serendipitously as a result of natural cellular labeling made possible by the atmospheric Cold War atom bomb testing that dramatically increased the level of C-14 in atmospheric $CO_2$. Although there is constant turnover of carbon atoms in all living cells, this does not apply to the DNA in the nucleus of the cell which once formed at the time of cell division and DNA replication, the carbon atoms in the DNA molecules remain stable. Since the half-life of C-14 is 5730 years the level of radioactivity is essentially constant throughout one's lifetime. Knowing the percentage of C-14 in atmospheric $CO_2$ that has decreased dramatically since its peak activity which occurred at the end of atmospheric bomb testing in approximately 1966, (see C-14 atmospheric activity curve) one can accurately determine the cellular age by analyzing the ratio of C-14 to the nonradioactive commonly occurring isotope C-12. Comparing this to the person's age, one can easily calculate the cellular turnover rate for that particular tissue.[29]

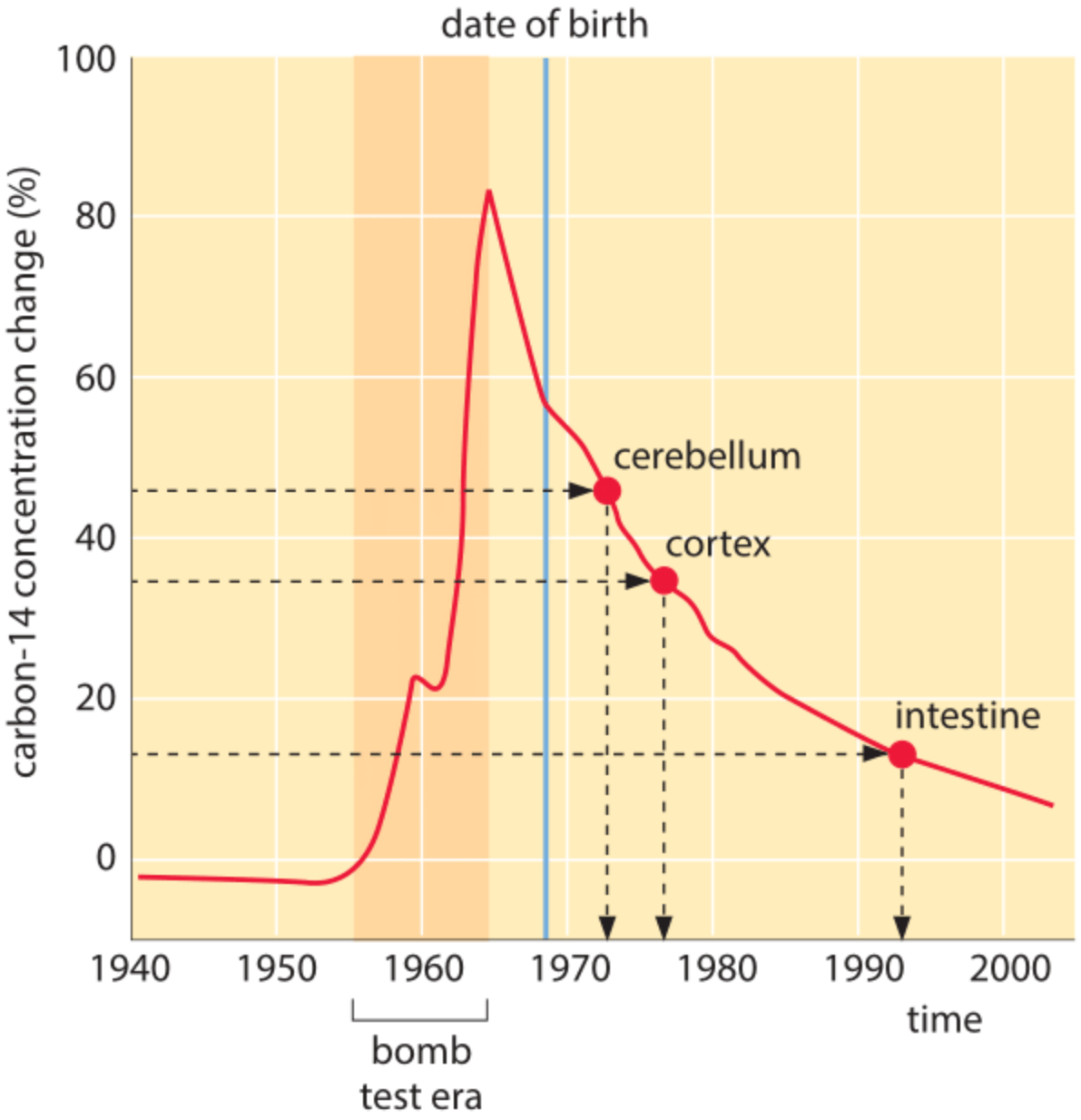



**Figure 15. Atmospheric $CO_2$ Carbon-14 Activity Curve Resulting from Atmospheric Atom Bomb Testing.**

| cell type | turnover time | BNID |
|---|---|---|
| small intestine epithelium | 2-4 days | 107812, 109231 |
| stomach | 2-9 days | 101940 |
| blood Neutrophils | 1-5 days | 101940 |
| white blood cells Eosinophils | 2-5 days | 109901, 109902 |
| gastrointestinal colon crypt cells | 3-4 days | 107812 |
| cervix | 6 days | 110321 |
| lungs alveoli | 8 days | 101940 |
| tongue taste buds (rat) | 10 days | 111427 |
| platelets | 10 days | 111407,111408 |
| bone osteoclasts | 2 weeks | 109906 |
| intestine Paneth cells | 20 days | 107812 |
| skin epidermis cells | 10-30 days | 109214, 109215 |
| pancreas beta cells (rat) | 20-50 days | 109228 |
| blood B cells (mouse) | 4-7 weeks | 107910 |
| trachea | 1-2 months | 101940 |
| hematopoietic stem cells | 2 months | 109232 |
| sperm (male gametes) | 2 months | 110319, 110320 |
| bone osteoblasts | 3 months | 109907 |
| red blood cells | 4 months | 101706, 107875 |
| liver hepatocyte cells | 0.5-1 year | 109233 |
| fat cells | 8 years | 103455 |
| cardiomyocytes | 0.5-10% per year | 107076, 107077, 107078 |
| central nervous system | life time | 101940 |
| skeleton | 10% per year | 109908 |
| lens cells | life time | 109840 |
| oocytes (female gametes) | life time | 111451 |

**FIGURE 16. RELATIVE CYCLING RATES TISSUES LIVING ORGANISMS**

### Tools for Observation: Compound Light Microscope

Our ability to understand living organisms has progressed in direct relationship to our progress in improving instruments of observation. In 1590 Zacharias Janssen and his father Hans devised the first compound microscope composed of a convex objective and a concave eyepiece. They were both Dutch spectacle makers who recognize that two lenses could be

arranged in a tube in such a manner that an object placed at the end of the tube would appear markedly enlarged, much bigger than could be achieved with a single magnifying glass. This was the first known compound microscope.

Galileo Galilee heard of their experimenting and began investigating on his own. He soon recognized the advantage of combining two lenses in tandem and independently devised both the compound microscope and telescope. The term "microscope" was first coined by Giovanni Farber in 1625 to describe the instrument invented by Galileo in 1609. His success with the telescope is well known. The four major moons of Jupiter still carry his name following his discovery that they were distinct satellites of Jupiter in March 1610.

In 1611 Johannes Kepler suggested the design of a compound microscope that was actually constructed by Christoph Scheiner in around 1628. This microscope has become the prototype for modern light microscopes.

In 1663 Robert Hooke, a contemporary of van Leeuwenhoek, first described cellular structures in plants. He was able to extend the range of vision of small structures by looking through globular glass lenses he devised. When studying a thin slice of cork, he was able to see many small air spaces that he called cells. He was the first person to observe living cells in living plant stems which he described in his *Micrographia* in 1665.[30]

In 1673, Anton Van Leeuwenhoek constructed many microscopes, more than 500, each consisting of a single lens with relatively high magnification. Using a single lens largely eliminated the problems of optical aberration. It was a simple portable device allowing him to make direct observations in the field. He was a Dutch draper and initially used magnifying glasses to study the threads in fabrics. He was able to achieve magnifications up to 270 times. Although he had no formal scientific training, he possessed a curious mind and reported his observations with diagrams and descriptive notes. He also observed living cells. He was able to observe bacteria, red blood cells, spermatozoa, microscopic structure of seeds, bones, skin, fish scales, oyster shell, tongue, nerves, muscle fibers, fish circulatory system, insect eyes, parasitic worms, spider physiology, mite reproduction, aquatic plants and the "animalcula" (a term he applied to the various protozoa) which he described in 1683 in a communication with the Royal Society.[31] He communicated his findings to the Royal Society in a series of letters

(Leeuwenhoek 1800 *The select works of Antony Van Leeuwenhoek, containing his microscopical discoveries in many of the works of nature*, volume 1). He has become known as "The Father" of microscopy.

In 1835 George Airy, based on his studies of wave phenomena of visible light that occurs when visible light passes through a circular aperture such as those associated with the lenses of a light microscope, recognized the "point spread function" (PSF). He mathematically described the pattern called an Airy Disk, which contains a central peak of light intensity surrounded by dimmer rings moving away from the center, forming concentric rings such as displaying by a target. The size of the areas of bright and dim light are directly related to the wavelength of the light.[32]

Ernst Abbe discovered that the limit on the size of the Airy disk was roughly half the wavelength of the imaging light which when so applied to the compound light microscope defined the maximum theoretical resolution as the wavelength of the incident light.

There have been many refinements to the early compound light microscopes such as binocular microscopes, phase contrast microscopy, transillumination, ultraviolet microscopy, (with its shorter wavelength that improves resolution) and special techniques utilized in chemical microscopy including crystallography. The useful magnification factor of modern light microscopes is limited to about 2000 times.

**Invention of the Electron Microscope**

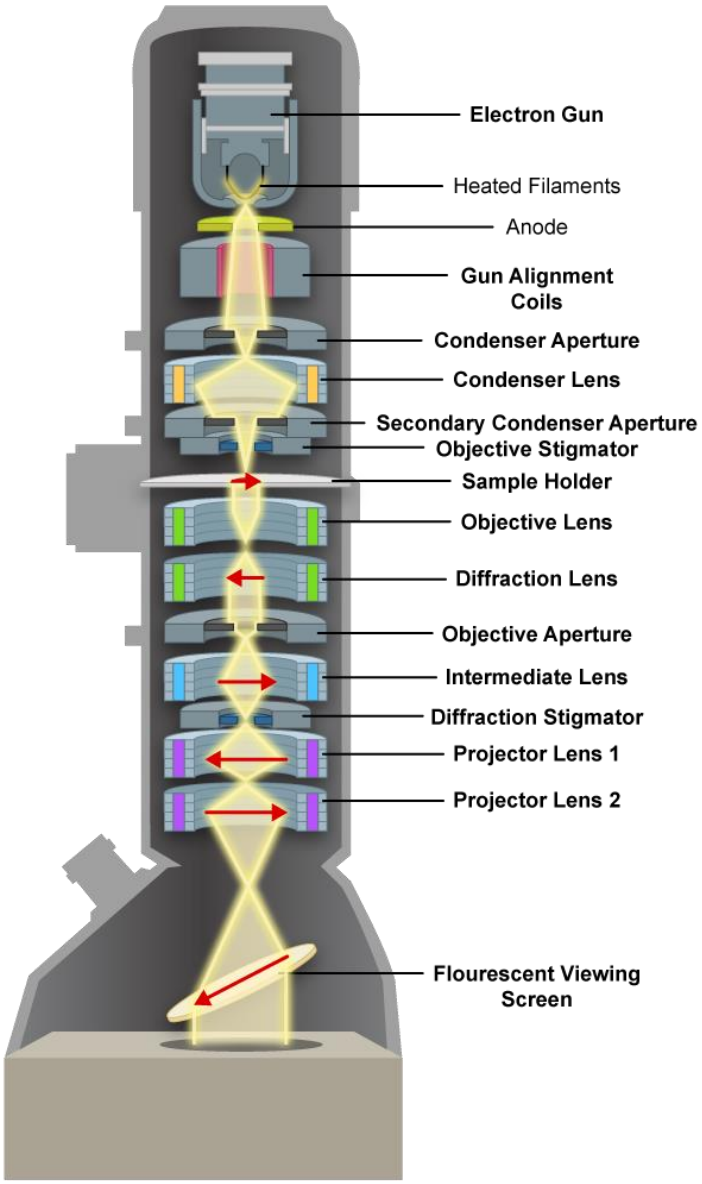
The next big step forward in improving our tools of observation of small objects was the electron microscope. In 1926 Hans Busch envisioned the possibility of microscopic imaging in which the illuminating light source is replaced by an electron beam. It was well known that the wavelength of the illuminating light is the limiting factor of the light microscope resolution. The much shorter wavelength of an electron beam can potentially increase the resolving power of the electron microscope over a light microscope by three orders of magnitude. Based on the work of Louis de Broglie and Erwin Schrodinger, and his own 15 years experience working with the effect of magnetic fields on the trajectories of electrons, he recognized that the application of a magnetic field serving as a lens could provide the basis for a microscope with markedly increased resolution.

Ernst Ruska, after reading a paper by Busch in which he described the effects of a magnetic field in directing electron beams analogous to the way light is refracted by optical lenses, recognized this as the basis for the development of the transmission electron microscope (TEM).[33] Although the patent for the electron microscope is held by Reinhold Rutenberg in 1931, the invention of the electron microscope is traditionally credited to the German physicist Ernst Ruska along with Max Knoll in 1931. Two years later Ruska built the first electron microscope which gave better resolution than a light microscope and in 1986 he was awarded the Nobel Prize for his work in the field of electron optics as well as his work in constructing the first electron microscope.


**Figure 17. Inventor of the electron microscope**

By 1940 commercially produced transmission electron microscopes (TEM) were available with relevant resolutions in the range of 20-24 Å. Since further refinement has increased the resolution to the range of 2 to 3 Å. It is of interest to note that in 15 years, the electron microscope has achieved a level of perfection comparable to that of the compound light microscope that required 300 years.[34]

## Figure 18. Diagram of an Electron Microscope




1. Electrons are emitted into a vacuum tube by heating *Cathode Filaments* in the electron gun
2. The cathode ray then passes through an *Anode*, which accelerates and focues the beam; *Alignment Coils* additionally accelerate the beam
3. An adjustable *Condenser Aperture* prepares the beam for the *Condenser Lens* by blocking off-axis or off-energy electrons from proceeding.
4. The magnetic *Condenser Lens* applies a magnetic field, inducing a helical path for the electrons, and leading the cone-shaped electron beam to converge on a spot
5. A *Stigmator* helps to adjust the beam and prevent astigmatism (different foci between rays) in the optical system
6. Electrons pass through the thinly sliced sample, inserted onto a grid-like stage
7. The *Objective Lens* focuses the image of the sample
8. A *Diffraction Lens* is used to apply Bragg Scattering to the electrons
9. The *Objective Aperture*, positioned on the back focal plane of the scattered rays, selects (or excludes) the portion of the sample that produced the scattering
10. *Projector Lenses* calibrate the magnification of the image
11. The image is visualized through oculars or by an image recording system underneath the *Fluorescent Screen*

### Other important types of electron microscopes

The transmission electron microscope (TEM) is by far the most commonly used type of electron microscope by microbiologists. A major advance in electron microscopy, particularly for the imaging of biological organisms was achieved by cryo-fixation. Cryo-fixation is a method of stabilizing a biological sample by near instantaneous freezing. The sample is plunged into a cryogen such as ethane which produces “virtuous ice” that forms so quickly that the water molecules do not have time to rearrange into a crystalline lattice which could cause distortions of the protein molecules. The low

temperature is maintained by liquid nitrogen, providing significant protection from the damaging ionizing effects by an electron beam. Once the specimen is fixed, it is maintained at a temperature below -195°C in liquid nitrogen until scanning is completed. This method preserves cellular and molecular structures in their living state. Cryo – EM contributed greatly to improved imaging of biological material particularly proteins that do not crystallize and therefore cannot be studied by x-ray crystallography. It has expanded the applications of transmission electron microscopy to the extent that the scientists who pioneered this technology, Jacques Dubochet, Joachim Frank and Richard Henderson received the Nobel Prize in chemistry in 2017.

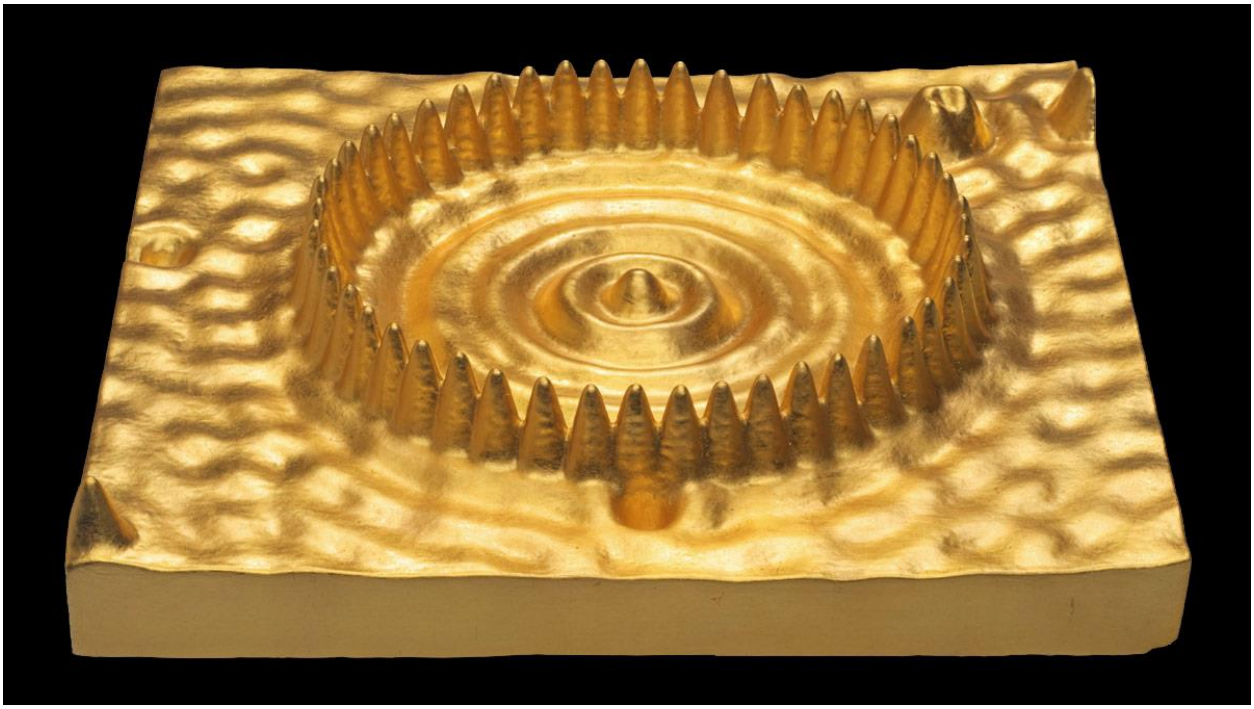
By maintaining a low temperature environment during scanning, the frozen hydrated biological specimen can be preserved in its native structural integrity even in a microscopic vacuum. At room temperature the water vaporizes almost immediately in the deep vacuum environment and the specimen is quickly desiccated. The low temperature also enhances the tolerance of electron exposure reducing the extent of radiation damage. The scanning time is so rapid that even if bonds are severed, the image of the molecule can be acquired and recorded before significant structural distortion can occur.

With the recent development of direct electron detectors that replace the older fiber-optic coupled scintillator – CCD technology, the speed of image detection and processing now matches the speed of data acquisition by Cryo EM imaging devices. The frame rate can essentially stop electrons in motion eliminating motion artifacts. The new detectors are capable of counting single electrons. Coupled with Movie Mode Imaging results in the elimination of motion artifact with rates of 10 to 40 per second.

Another major type of electron microscopic imaging is the Scanning Tunneling Electron Microscope (STM) for which Binnig and Roher shared the Nobel Prize in physics in 1986. This device is capable of imaging individual atoms and is used extensively in material research in which the crystalline formation of metals and their alloys can be analyzed at the atomic level. A striking image demonstrated by this means displays 48 atoms of iron sitting on a sheet of copper displayed in a circle configuration known as a quantum corral.

**Figure 19. Tunneling Electron Microscope Image, Quantum Corral**

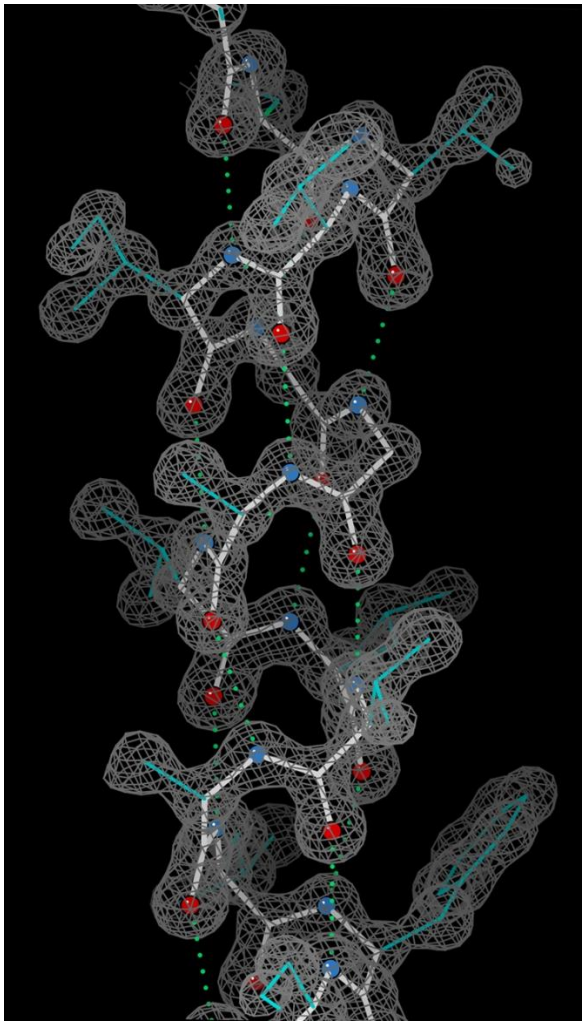
**This image displays 48 iron atoms forming a quantum corral positioned on a copperplate**



## X-ray Crystallography

X-ray crystallography is based on diffraction of electrons by crystal lattices. In biochemistry it is limited to compounds that crystallize. It produces precise, high-resolution images of atoms. It has contributed greatly to our understanding of biological molecular structures.

**Figure 20. The image below displays the structure of the protein alpha helix molecule with stick figures for the covalent bonding within the electron density for the crystal structure. The image is performed at high-resolution (0.91 Angstrom). The density contours are in gray, the helix backbone in white, the sidechains in cyan, oxygen atoms are in red, nitrogen atoms in blue, and hydrogen bonds are dotted green lines.** (Courtesy of protein databank file, 2NRL, residues 17 – 22)



**In Conclusion:**

This does not in any way cast criticism on Darwin's insights into the relationship of evolutionary origins and what he observed in his concept of the tree of life. But it illustrates a fundamental aspect of true scientific investigation; i.e. the responsibility of scientists to remain alert to how new knowledge and understanding must be constantly applied to theoretical understandings or propositions based on past knowledge.

How is it possible for modern-day scientists who are now fully cognizant of the complexity and evidence that has essentially overwhelmed OOL scientists in recent decades in terms of the irreducible complexity of living organisms at the molecular level to continue to assume that the pathway of prebiotic chemistry leading to simple biology still represents a plausible approach to the OOL? The recognition that all living cells function only in an environment of a resting transmembrane electrical potential that is based on

complex nanomachines that could not possibly have occurred by random, gradual, mutations and resultant adaptation based on survival of the fittest, should intellectually be deterrent enough. There is no conceivable way based on present knowledge that the nanomachines needed to produce the energy required to power the sodium potassium ion pump, or the synthesis of the ribosome required to build the nanomachines utilizing only L-stereospecific amino acids, or an exclusive source of D-stereospecific ribose and the series of RNA polymerases needed to generate the mRNA that carry instructions accurately from nuclear DNA to ribosomes, could all have by gradual random mutations achieved the complexity and synchronous functionality from which the first living cell could have emerged.

And there just hasn't been enough time for the survival of the fittest approach to solve the combinatorial problems of complex molecular structures. Nor is there scientific substantiation that the process of random mutations could bring about the amazing evolutionary developments that surround us on every side. Most genetic mutations are seriously detrimental and many incompatible with life. It is estimated that 20 to 30% of all human pregnancies terminate in spontaneous abortion due to genetic defects incompatible with life. I have personally observed this phenomenon during my many years of practice of obstetrical ultrasound.

There is the Cambrian explosion during which all the phyla emerged during the blink of an eye of geological time. There are many other unexplained timing issues that are incompatible with neo-Darwinian theory.

And finally, there is the unsurmountable dilemma, to resolve the origin of the **universal genetic code**. Our search here is for the source of the information universally encoded into the living genomes of all the organisms that inhabit the planet in which the same three letter genetic word encodes the same amino acid whether it is found in plants of 400 million years ago, reptiles of a hundred million years ago, in mammals of 50 million years ago or in humans of one-half million years ago. In doing so we are also searching for the foundation on which the **central dogma of molecular biology** is based.

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