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# CHEMICAL PERSPECTIVES ON THE ORIGIN OF LIFE AND THE CREATION OF SYNTHETIC CELL

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## Abstract

One of the existential questions that scientists have pondered since antiquity, i.e. the question of life's primordial beginning, belongs directly in the domain of Chemistry. It is therefore relevant to speculating on and studying the kinds of chemical reactions that would have been important in molecular origins. The origin of life can be addressed in the platform of interdisciplinary approach that covers chemical conundrums as well as biological, physical and philosophical sciences. In this article we focused on the chemical perspectives on the origin of life and the creation of synthetic cell because of its overwhelming chemical significance. We draw our attention to the creation of the world's first synthetic cell and this study on creation of life and the underlying chemistry intelligibly underlines that while creating the new life forms or modifying the existing one, we should reveal our concern towards the future generation the environment concerned. The study illustrates that the creation of life and related scientific discoveries need to focus primarily on societal benefit by rendering respectful approach to human dignity. However, despite the years scientists are unable to create life through chemical synthesis or by using other sophisticated technologies. This article also highlights the chemoautotrophic origin of life on Earth and the 21st century perspectives of life. The uncertainties pertaining to the origin of life are adequately addressed in the article. We conclude that the origin of life from non-living molecules in the prebiotic environment is still obscure to our knowledge and investigations and the creation of fully functional cell is not yet a reality. For the critical analysis of the origin of life and creation of synthetic cell from the chemical perspectives, we applied biochemical and analytical methods which derives ultimate ground of hope from chemical resources.

*Keywords:* societal benefit, human dignity, biochemical, analytical, methods

## 1. Introduction

Life is an incredibly complex unsolved mystery and the creation of life has been a big puzzle despite rapid advancements in the field of Synthetic biology, Microbiology and Chemical sciences in recent years. The questions pertaining to the chemistry of life can go very deep. Why did nature choose phosphates found in DNA (Deoxyribo Nucleic Acid) and ATP (Adenosine Tri Phosphate), the energy currency of the cell? Why α-amino acids rather than β-

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amino acids? Why ribose and deoxyribose sugars? These questions lead us to basic discussions of nucleophilicity, steric effects, thermodynamics, kinetics, atomic sizes and myriad other fundamental concepts in Chemistry. The great advantage of Chemistry is that through its synthetic and creative capabilities it can actually *vary* the fundamental properties of life's molecules and ask what the consequences should be. The culmination of this capability is the exciting science of Synthetic biology whose practitioners are already investigating the effects of creation of synthetic cell by inserting artificial DNA and non-standard amino acids on biological function. The DNA is considered to be the building block of life and the methods of isolation and manipulation of DNA to understand their roles in the development and functioning of the cell have enhanced enormously since their inception in the 1970's. The growing accuracy of these methods has allowed scientists to develop and refine such tools as DNA fingerprinting, artificial cell membrane, disease-resistant crops, and tests for heritable diseases. Recently, by inserting some extra genes into the harmless strain of *Escherichia coli* bacteria, scientists at the Rensselaer Polytechnic Institute, New York have produced anthocyanins [D. Charles, *Who made that flavor? Maybe a genetically altered microbe*, 4 December 2014, http://www.npr.org/blogs/2014/12/04/who-made-that-flavor-maybe-a-genetically-altered-microbe?], the powerful antioxidants that scavenge free radicals produced through metabolic processes [1-3]. Currently, researchers are actively involved in search for the origin of life by synthesizing and manipulating the genetic materials and other cell organelles.

## 2. Early Earth and beginning of life

Earth formed as part of the birth of the Solar system about 4.6 billion years ago and it was then very different from the world known today. There were no oceans and oxygen in the atmosphere and during the period 4.3–3.8 billion years ago, it is believed to have undergone a period of heavy meteoric bombardment for about 700 million years. This bombardment combined with heat from the radioactive breakdown and heat from the pressure of contraction made the planet at this stage to be fully molten and heavier elements sank to the centre while the lighter ones rose to the surface producing Earth's various layers. The early earth was lifeless and simply inhospitable with its atmosphere dominated by light gases such as hydrogen and helium. The planet is believed to have cooled quickly and the solid crust was formed within 150 million years and formation of clouds in about 200 million years. The subsequent rains gave rise to the oceans and making it an inhabitable planet for the first time in its history within 750 million years of Earth's formation. Liquid water is the most essential ingredient to trigger the beginning of life and water provides an excellent environment for the formation of complicated carbon-based molecules that could eventually lead to the emergence of life. Steam escaped from the crust, while more gases were released by volcanoes, creating the second atmosphere in Earth's early history. Life on Earth may have emerged during or shortly after the early heavy bombardment phase, perhaps as early as 3.90–3.85 billion years ago, but the precise timing remains uncertain. It is generally believed that until 2.4 billion years ago, the Earth's atmosphere was generally devoid of oxygen. Volcanic activity was intense and without an ozone layer to hinder its entry, ultraviolet radiation flooded the surface. Thus, the early Earth was just one big chemical factory [4].

The Earth was covered in a hot, thin soup of water and organic materials (primordial soup) and the molecules became more complex and began to collaborate to run metabolic processes. Eventually, the first cells came into being and these cells were heterotrophs, which could not produce their own food and instead fed on the organic material from the primordial soup. Heterotrophs are organisms that obtain their energy by *feeding* on *others* (or on organic compounds). The anaerobic metabolic processes of the heterotrophs released carbon dioxide into the atmosphere, which allowed for the evolution of photosynthetic autotrophs, which could use light and $CO_2$ to produce their own food. The few heterotrophs that survived the change in environment generally evolved the capacity to carry out aerobic respiration and over the subsequent billions of years; the aerobic autotrophs and heterotrophs became the dominant life-forms on the planet and evolved into all of the diversity of life now visible on Earth [5].

## 3. Chemoautotrophic origin of life

According to the chemoautotrophic origin of life it is suggested that life started autotrophically and that the oxidative formation of pyrite ($FeS_2$) satisfies all the necessary conditions to be met by an energy source for such an origin. The most commonly cited autotrophic hypothesis stems from the work of Gunter Wächtershäuser [6], who has argued that life began with the appearance of an autocatalytic two-dimensional chemolithotrophic metabolic system based on the formation of the highly insoluble mineral pyrite ($FeS_2$). The $FeS/H_2S$ combination is a potentially strong reducing agent, and has been shown to provide not only an efficient source of electrons for the reduction of organic compounds under atmospheric pressure and temperatures below 100°C, but also to promote the formation of peptide bonds by activation of amino acids with CO on (Ni, Fe)S surfaces, as well as the fixation of carbon monoxide into activated acetic acid by a mixture of co-precipitated NiS/FeS [7].

These results are compatible with a general, modified model of a 'primitive soup' in which pyrite formation is recognized as an important source of electrons for the production of reduced organic compounds.

## 4. Prebiotic chemistry

Biomolecules are the precursors for the origin of life and are formed by numerous chemical reactions that might have happened in the primordial Earth. The Strecker reaction for amino acid synthesis and formose reaction for carbohydrate synthesis have been known as the front runners for the genesis of biomolecules.

The Strecker amino-acid synthesis involves the synthesis of amino acid from carbonyl compounds such as aldehyde or ketone in a series of chemical reactions. In the first step, aldehyde condensed with ammonium chloride in presence of potassium cyanide to form an alpha aminonitrile, which is further subjected to hydrolysis to give the desired amino-acid [8].

The formose reaction involves the synthesis of sugars from formaldehyde and as far as the question on the origin of life is concerned, the formose reaction is of great importance as it explains part of the path from simple formaldehyde to complex sugars like ribose and from there to RNA (Ribo Nucleic Acid). The reaction starts with the condensation of two molecules of formaldehyde to make glycolaldehyde which further reacts in an aldol reaction with another equivalent of formaldehyde to make glyceraldehydes. This is followed by the formation of aldotetroses and this reaction is highly useful for the synthesis of ribose sugar, the main constituent of RNA [9].

Proteins play a significant role in almost all biological processes and amino acids are the building block of it. These reactions are plausible in the early earth conditions and the organic compounds such as amino acids, carbohydrates and other complex molecules formed by the chemical reactions paved the way for the origin of life on primitive Earth.

## 5. Modern theory of origin of life

According to the modern theory of origin of life, proposed independently by Oparin and Haldane, the first life forms were unable to synthesize their own compounds, but were formed from and dependent on pre-existing organic compounds of abiotic origin [10]. It also stated that primitive life originated in the water bodies on the primitive Earth from non-living organic molecules such as RNA, proteins, etc. by chemical evolution through a series of chemical reactions about 4 billion years ago. This theory has been experimentally tested by Stanley Miller and Harold Urey [11] and the synthesis of organic compounds under primordial Earth conditions was accomplished by electric discharges acting on a mixture of ammonia ($NH_3$), methane ($CH_4$), hydrogen ($H_2$) and water ($H_2O$); racemic mixtures of several amino acids, hydroxy acids, urea and other organic molecules were produced [12]. These experiments follow a generalization that if this can happen in a lab, it could have occurred in a similar way on the primitive earth atmosphere. Miller’s experiment was followed a few years later, Or´o and Kimball synthesized adenine, the nitrogenous base in DNA, by the polymerization of HCN under basic conditions [13]. The role of HCN in prebiotic chemistry has been further supported by the discovery that hydrolysis of HCN polymers yields several amino acids, purines and orotic acid, which is a biosynthetic precursor of the pyrimidine uracil, a constituent of RNA [14]. The ease of formation of amino acids, purines and pyrimidines from simple precursors under prebiotic conditions strongly suggests these components were present in the prebiotic environment.

As is well known, all aspects of cell formation are directed by the base pair sequences in DNA. No laboratory simulations have been able to produce even a small segment of a DNA molecule or even the simplest proteins without first seeding the experiment with DNA. Stanley Miller's classic experiment in 1955 demonstrated that simple amino acids and sugars can be synthesized from a random mixture of gases in a reducing atmosphere exposed to electrical discharges [15]. However, the macromolecular structures characteristic of life cannot be produced by such nondirected synthesis [16]. Though scientists have synthesized various amino acids in the lab by chemical processes under primordial earth conditions, there is a definite gap exists between amino acids and living beings. Amino acids are the monomer unit of protein molecule; and it does not indicate life as they are not living molecules.

## 6. Molecular self-assembly

The molecules formed by the chemical reactions in the early Earth might have held together by hydrogen bonding and other electrostatic interactions eventually led to the accumulation and formation of more complex molecules such as fats, nucleotides, nucleic acids and polypeptides. These complex organic compounds synthesized on the primitive Earth later tended to accumulate and formed cell-like large colloidal aggregates called protobionts [17]. These colloidal aggregates are giant molecules containing RNA, protein, polysaccharides, etc. and such first non-cellular forms of life originated probably three billion years back.

How life originated from the non-living biomolecules through series of chemical reaction and how the first life form came into existence are the serious questions to be answered. The complex organic biomolecules (proteins, polysaccharides, RNA, etc.) formed from simple molecules by chemical pathways and subsequent electrostatic interactions do not symbolize life. Though researchers synthesize biomolecules in the lab by establishing the primordial Earth conditions, the origin of life from such a non-living molecule still remains as a mystery beyond our comprehension.

## 7. Origin of life - an unsolved puzzle

How living organisms came into existence out of nonliving matter was an issue that evolutionists did not even want to mention for a long time. However, this question, which had constantly been avoided, eventually had to be addressed, and attempts were made to settle it with a series of experiments in the second quarter of the twentieth century. The main question was: How could the first living cell has appeared in the primordial atmosphere on the Earth?

The first person to take the matter in hand was the Russian biologist Alexander I. Oparin and despite all his theoretical studies, Oparin was unable to produce any results to shed light on the origin of life. He says the following in his book *The Origin of Life*, published in 1936: "Unfortunately, however, the problem of the origin of the cell is perhaps the most obscure point in the whole study of the evolution of organisms" [18].

Since Oparin, evolutionists have performed countless experiments, conducted research, and made observations to prove that a cell could have been formed by chance. However, every such attempt only made the complex design of the cell clearer, and thus refuted the evolutionists' hypotheses even more. Professor Klaus Dose, the president of the Institute of Biochemistry at the University of Johannes Gutenberg, states: "More than 30 years of experimentation on the origin of life in the fields of chemical and molecular evolution have led to a better perception of the immensity of the problem of the origin of life on earth rather than to its solution. At present all discussions on principal theories and experiments in the field either end in stalemate or in a confession of ignorance." [19]

The following statement by the geochemist Jeffrey Bada, from the San Diego-based Scripps Institute, makes the helplessness of evolutionists clear: "Today, as we leave the twentieth century, we still face the biggest unsolved problem that we had when we entered the twentieth century: How did life originate on Earth?" [20].

About 3.8 billion years ago, life appeared on Earth in the form of bacteria, simple cells without a nucleus (*prokaryotes*). But then something dramatically new happened. The *eukaryotes* appeared. There is evidence of life's signature found in the carbon of rocks from Greenland dated at 3.85 billion years old. It is not known where or how life began [21].

Despite decades of work, origin-of-life theorists are at a loss to explain how this system arose. In 2007, Harvard chemist George Whitesides was given the Priestley Medal, the highest award of the American Chemical Society. During his acceptance speech, he offered this stark analysis, reprinted in the respected journal Chemical and Engineering News: "The Origin of Life. This problem is one of the big ones in science. It begins to place life, and us, in the universe. Most chemists believe that life emerged spontaneously from mixtures of molecules in the prebiotic Earth. How? I have no idea." [22]

Many other authors have made similar comments. Massimo Pigliucci states: "It has to be true that we really don't have a clue how life originated on earth by natural means" [23].

Science writer Gregg Easterbrook wrote in Wired, "What creates life out of the inanimate compounds that make up living things? No one knows. How were the first organisms assembled? Nature hasn't given us the slightest hint. If anything, the mystery has deepened over time." [G. Easterbrook, *Where did life come from?*, 15 February 2007, 108 http://www.evolutionnews.org/2012/04/on_the_settled_058221.html]

It is quite evident from the views of the scientists that the constraints of historical science are such that the origin of life may never be understood [24]. How life originated from the mixture of molecules in the primitive Earth is obscure to our knowledge as the spontaneous emergence of life from the mixture of molecules is not realized even today. The advances in chemical and biological sciences enable the scientists to synthesize biomolecules, the precursors of the origin of life. But, how life originates from these inanimate molecules by chemical processes or any other phenomenon is yet to be answered.

## 8. What is life? - a 21st century perspective

The insatiable quest for the origin of life continues with a fuzzy thinking on the emergence of life and neither Chemical science nor any other emergent technologies give satisfactory explanation for the life's primordial beginning from non-living molecules. Likewise, the creation of life is also an unattainable task since ancient times and the emergence of new technologies prompted scientists to synthesize life in the lab. However life is an unexplained mystery over the years and the 21st century approach on life focused mainly on the synthesis, transplantation and manipulation of DNA. Till 1944, the world had no idea about what the genetic material was and scientists thought it was proteins, not DNA. In 1949, the protein insulin was sequenced by Frederick Sanger and this work showed that proteins consisted of linear amino acid codes [25]. The sequence of insulin was very crucial in terms of leading to understanding the link between DNA and proteins. The next big thing came from Gobind Khorana and Marshall Nirenberg [26] they worked out the triplet genetic code, three letters of genetic code, coding for each amino acid. This was followed by Robert Holley's discovery of the structure of transfer RNA (tRNA) that carries amino acids from the cytoplasm of the cell to the site of protein synthesis [27].

The 1970s brought the beginning of the molecular splicing revolution [28] and using restriction enzymes Cohen and Boyer, and Paul Berg developed recombinant DNA [29] and this technology was used to produce human insulin as the first recombinant drug [30]. In 1995 the decoding of the first genome from the bacterium *Haemophilus influenzae* Rd was carried by Fleischmann and co-workers [31] and this is followed by the decoding of human genome by Craig Venter [32]. In 2006, Jay Keasling at the Lawrence Berkeley National Laboratory in California inserted a group of genes into yeast to produce a precursor for an anti-malarial drug [33]. In 2010, Craig Venter and team [34] created a living cell made from four bottles of chemicals and it further confirmed that life is a DNA software system and this is one of the landmark events of 21st century science.

Synthesis of cell is the primary step in the creation of life because cells are the basic units of life. Cells are made up of numerous molecules and they perform various metabolic processes essential for life. Hence synthesis of a living cell is a milestone in the scientists' search for the creation of life.

## 9. Synthetic cell

By synthesizing cell in the lab, scientists headed one step forward in their attempt to create life. The synthesis of living artificial cell is not an easy task as none of the chemical processes or scientific advances provide reliable evidences for the creation of a living cell. A living artificial cell is a synthetically made cell that has the capacity to capture energy, maintain ion transport and contain macromolecules as well as store information [35]. In 2010, Craig Venter and team synthesized a variation of an artificial cell that has been created by the transplantation of synthetic genome of a bacterium into a genetically emptied host cell [34]. This man-made cell is not completely artificial because the synthesized genome is transplanted into a living cell and the genome made use of the cellular machinery of the host cell.

Thomas Chang at McGill University developed the first non-living artificial cells in the 1960s [36] and later, researchers introduce enzymes, proteins and hormones to artificial cells leading to clinical use in diseases such as Lesch-Nyhan syndrome [37]. Biodegradable artificial red blood cells were developed in the mid-1990s and artificial cells in biological cell encapsulation were first used for the treatment of diabetes [38]. The creation of an artificial cell membrane was reported by chemists at Havard University [39] and artificial eukaryotic cell capable of performing chemical reactions through working organelles were synthesized in 2014 [http://phys.org/news/2014-01-plastic-cell-organelle.html]. Researchers have successfully developed artificial cell organelles that are capable of supporting the reduction of toxic oxygen compounds in the cell and this opens up new horizons in the development of novel drugs [40].

Advances in Genetic engineering allow the expression of many genes, but the efforts are far from producing a fully operational cell. Although the science is advancing with unimaginable pace, the creation of a fully functional cell is hitherto not a reality.

## 10. Creation of synthetic cell controlled by chemically synthesised genome

Scientists from John Craig Venter Institute (JCVI), California have created the world's first self-replicating synthetic cell completely controlled by man-made genome. The complete DNA sequence of the bacterium *Mycoplasma mycoides* was successfully designed in the computer using a natural genome as a template and it was brought to life through chemical synthesis. The genome was synthesized chemically in many pieces, stitched together by means of molecular biological techniques and transplanted into the cytoplasm of an existing bacterial cell. The donor and recipient bacterial cells are of the same genus *Mycoplasma,* so as to facilitate the correct protein interactions. *Mycoplasmas* are small parasitic bacteria that can cause human respiratory and inflammatory diseases. The synthetic genome of *Mycoplasma mycoides* was transplanted into the bacterium *Mycoplasma capricolum* that had its DNA removed. The genome took the control of the host cell and started divided over to billions of *Mycoplasma mycoides* cells [34]. DNA is the template for protein construction and requires proteins as helper molecules to do so; consequently synthetic naked DNA would require several proteins to create a viable cell. The synthetic genome of *Mycoplasma mycoides* replicates by utilizing the cellular machinery of the host cell.

Deoxyribo Nucleic Acid (DNA) is made from large number of molecular components and the chemistry of these building blocks reveals that DNA does not represent life. The synthetic DNA is a non-living molecule and it requires a living environment to function. So the creation of synthetic cell by Craig Venter and team can be regarded as the modification of life rather than creation. Though the researchers were able to synthesize artificial cell membrane, DNA, artificial organelles and even artificial intelligence, they could not give life to non living molecules. Scientists had begun their attempt to create life since ancient times, but the creation of life is still a mystery to be solved despite of the advanced technologies.

This creation of synthetic cell was a remarkable feat in the field of Synthetic biology as this technology can be utilized to engineer microbes for environmental or medical applications. Nevertheless, releasing a new life form into the environment raises serious questions regarding the safety and security of the people and the environment concerned. Therefore, the creation of synthetic cell requires the careful development of ethical framework as well as the ongoing assessment of its potential risk, utility and impact on society.

## 11. Synthetic cell - challenges/possibilities in the contemporary times

This creation of artificial life will open the way to creating useful microbes for the production of vaccines and biofuels [N. Wade, *Researchers say they created a synthetic cell*, 20 May 2010, http://www.nytimes.com/2010/05/21/science/21cell.html]. Among many promising application of this technology, two of the most encouraging are biofuel producers and cancer cell destroyers. Researchers are currently engineering algal cells to possess an enhanced genome that can produce range of biofuels including biodiesel, ethanol and hydrogen. These microorganisms produce fuels using only water, carbon dioxide, industrial waste and sunlight [41]. Recent research on the production of biofuel is being speculated with carbon-negative organisms and these organisms have more carbon intake than emissions, thereby reducing the green house gases and providing clearer fuels [D. Watts, *Synthetic Biology: An era of promised uncertainty*, 1 July 2010, http://www.biofuelreview.com/content/view/1493/].

Though synthetic biology encompasses a promised era of uncertainty, much more effective cancer therapies may soon be realized with the application of synthetic bacteria. Current cancer treatments indiscriminately attack both tumours and normal tissues, while inefficiently penetrating the former [42]. Genetically engineered *Escherichia coli* cells would first invade the body without alarming the immune system [43]. The cells would be programmed to find tumour tissues and the *E. coli* would implement their destructive force through a “cytotoxic or immunostimulatory response” [44], meaning they would attempt to destroy the tumour tissues by releasing toxic chemicals, or by triggering an immune response. Though the research is still ongoing, the introduction of synthetic bacteria will undoubtedly open the floodgates for the cancer treatment using these engineered microbes [45].

Creation of synthetic life, though beneficial, raises profound ethical concerns about the probable misuse of this emergent technology. The synthesis of genome of existing bacterium and the ability to create novel organisms invariably carries some threat that these organisms will behave in unexpected ways once they released into the environment. The overwhelming uncertainties concerning the creation of synthetic life led us to the critical analysis of Venter’s creation of synthetic cell. The analysis showed that scientists have created synthetic cell with laudable motives that paves the way for modified bugs that could revolutionize healthcare and fuel production. Scientists are not tampering with the essence of life rather they have been focused on addressing the societal implications of technology for the benefit of all. The biochemical analysis revealed that the creation of synthetic cell cannot be regarded as ‘creation of life’ because only the genome and none of the cytoplasmic structures were synthesized by scientists [46]. Also, Venter’s accomplishment was far from an act of creation or disproof of God and is morally justified. Though Venter’s team couldn’t completely eliminate the threats associated with ‘creating synthetic life’ or the abuse of this emerging technology; ongoing assessment of possible threats along with potential benefits could alleviate the plausible risks.

As the technology can be utilized for a dual purpose, the outcome depends upon not the tool itself, but upon the hand that executes it. For instance, Einstein’s famous theory of relativity that mass and energy are inter-convertible and are both but different manifestations of the same thing, led to the invention of atom bomb; the same principle can be utilized for the production of electric energy. Since the consequence of our action affect the future generation, it is of vital importance to make use of our prudence and wisdom. However, we need to foster the development of Science in a way that enhances its potential benefits while mitigating the probable risks and likelihood of direct and indirect harms. All involved have a responsibility to protect against negative effects resulting from new products to the environment. Responsible research guarantees a respectful approach to the dignity of the human person as well. The primary concern for all research should be to safeguard the earth’s bounty, the world’s safety, and the environment in which future generations will flourish.

## 12. Conclusions

The quest for the origin of life commenced since ancient times and the series of chemical reactions in the early earth might have produced non-living biomolecules essential for life’s primordial beginnings. Though the primitive earth conditions can be accomplished in the lab and water provides the ambient atmosphere to trigger life, nevertheless, scientists are still on the road to achieve the goal. Chemical reactions are of vital importance to sustain life on earth and the reactions that led to the origin of life from non-living molecules is still a mystery to be unveiled. Origin of life is literally a problem without end and we will not encounter a unique solution that satisfactorily explains the beginning of life at a molecular level. The origin of life has given us a perpetual inquiry that tosses out fundamental questions for us to discuss, debate and research.

Researchers are trying to synthesize fully functional cells and this will lead to the better understanding of life. However, despite the scientific advancements, the dream of the creation of autonomous synthetic life has not been realized till today. Life in its simpler form consists of enzymes, RNA and DNA molecules and other complex molecules enclosed within a very complex membrane. The various chemical reactions take place in the cell are strictly coordinated with one another towards the functioning of a living cell. The complex structure and function of the cell as well as its fabulous capabilities reveal a master plan.

The recent developments in chemical sciences and other emergent technologies couldn't explain the origin of life from molecules that are not alive and creation of life from nothing. Scientists are actively involved in serious search focused on the creation of life and also to unfold the mystery behind the origin of life. Perhaps it may be a reality in the future, albeit, the billions of years of enquiry fail to give answer to such a query. In other words, origin of life and creation of life are unsettled enigmas and hitherto no one is able to give precise explanations to these.

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