1. INTRODUCTION

One of the most outstanding contributions in the field of biological science is the discovery of DNA structure and the role of RNA displayed in the synthesis of protein. The spectacular success in the analysis and comprehensive understanding of genetic code and the nature of gene regulation laid foundation in the field of molecular biology. This molecular approach, in turn, has shown greater diagram of positive influence in understanding traditional disciplines such as genetics, cytology, embryology, and evolutionary biology.

Several refined work pertaining to molecular biology is based on the work carried out by bacterial experiments. One of the most simple and reliable organisms in strong thring the studies is Escherichia coli. Molecular biology is not only pertaining in understanding of biomolecular of prokaryotes but also eukaryotic cell. The fine tuning in understanding the cell function of higher organisms laid a foundation for the inventing strategies of genetic manipulation of cells and future cutting edge technology.

The primitive earth is believed to have formed about 4,800 million years back, as a hot fire ball without atmosphere. The cooling process of earth might have taken place for millions of years which resulted in random collision between neutrons and protons to form atomic nuclei. Further cooling resulted in binding of electrons to atomic nuclei. When accumulated gas inside the earth escaped slowly from the earth’s crust through surface cracks, it formed atmosphere. Water was formed immediately and entered the atmosphere due to evaporation. As the water recycled, continuous oceans were formed as a result of torrential rains for millions of years. In the beginning there was virtually no atmospheric oxygen on earth. The present day life entirely depends on the availability of oxygen on this planet which was originated millions of years back.

The nature and types of atmosphere on primitive earth was debated for a long time. Undoubtedly primitive earth comprised of a set of gases like hydrogen, methane, and ammonia. Hydrogen sulphide and carbon dioxide were probably present permanently as an essential component of primitive earth. The available condition on primitive earth promoted several chemical reactions to take place in the atmosphere as well as in the gases that dissolved in water. The energy for these reactions were derived from the sun radiation as well as from the electric discharge. Further, accumulated gas and volcanic eruptions discharged gases into the atmosphere.
The life is originated from primitive molecules million years back. However, there are fundamental questions on how did biomolecules arise? Biomolecules perhaps started interacting with each other and organised into specific function. It was amazing to presume how the first cell originated from these well organised biomolecules. Several decades of fundamental studies reveal adequate information on the possibilities of generating biomolecules under laboratory conditions.

1.1 ORIGIN OF ORGANIC MOLECULES

Primitive earth consisted of wider variety of important biomolecules at sufficient concentration and acted as building blocks to construct primitive life on this planet. The spontaneous prebiotic evolution suggested this view. During 1920 and 1930 considerable work has been done by J.B.S. Haldine and A.I. Oparin on the process of spontaneous production of nucleotides and primitive polymers.

Several pieces of evidence suggested that several organic molecules might have synthesized originally from abiotic origin on the primitive earth (primeval earth). The non-oxidizing earth’s atmosphere undoubtedly contained $\text{H}_2\text{S}$, $\text{NH}_3$, $\text{CO}_2$, $\text{CH}_4$, $\text{H}_2$ and $\text{N}_2$ except oxygen. The presence of sufficient concentration of precursor molecules promotes the synthesis of organic molecules using sun’s radiant energy and thermal energy provided by volcanic eruptions. The hydrogen was soon lost as a result of oxidised component. The oxygen appeared much later and its concentration built up gradually due to continuous photosynthetic activity on the earth.

1.1.1 Miller’s Experiment

Stanely Miller, a student of Harold urey, conducted series of interesting experiments in 1953, to demonstrate the synthesis of organic compounds under invitro conditions, in which he simulated possible prebiotic conditions prevalent on the primeval earth. He mixed methane, hydrogen and ammonia with water in a closed reflux vessel. Inside the reflux vessel the mixed gases which simulated the early atmosphere was exposed to electric discharges. The simulated primeval ocean contains more than 10 percent of the carbon (from methane) was included in the organic molecules like glycine, alanine, valine and leucine. Several intermediate products like hydrogen cyanide (HCN), aldehyde and cyano compounds were produced in the invitro experiments. These intermediates could have been an important intermediate in the formation of amino acids and nucleic acids. Miller’s experiments have demonstrated different reactions that mimic prebiotic conditions that are responsible for the synthesis of different aminoacids. He mixed hydrogen cyanide, aldehyde and cyano compounds with $\text{H}_2\text{S}$ and supplied ultraviolet light to provide energy for the reactions which lead to the formation of different aminoacids.

Invitro simulated experiments showed that reactions can be initiated by thermal energy released by volcanic eruptions and ultraviolet from solar radiation and electric discharge from lightening under simulated condition. Hydrogen cyanide in turn is the precursor for other reactive compounds such as cyanamide, cyanoacetylene and nitrites. These further act as fine precursors of various aminoacids, purine and pyrimidine. Heating the mixture of ammonia and hydrogen cyanide under simulated conditions of primeval earth results in the production of number of amino acids and considerable amount of purine adenine.
1.2 PREBIOTIC ORIGIN OF COMPLEX BIOMOLECULES

When primordial building blocks were formed from abiotic synthesis, further chemical organisation resulted in complex biomolecules. This must have been achieved by covalent bond linking the building blocks to form more complex molecules such as polysaccharides, polypeptides and polynucleotides. The covalent bond linkages between the building blocks can take place by condensation reactions which involves removal of water molecules. However, some apprehensions were raised about these linkages due to thermodynamically unstable condition of peptide, ester linkages and glycosidic bonds in aqueous condition. Hence, possibilities of small amount of such linkages can exist. Many speculations were followed in the process of condensation reactions. One is the involvement of chemicals in condensation reaction which was believed to extinct water molecules in the reaction facilitated covalent linkages. However, S.W. Fox, a leading investigator on origin of life threw light on the exact nature of chemical reaction. Covalent linkages which binds prebiotic polymers were largely thermal in nature. This presumption was evidenced when a polypeptide was formed by heating the mixture of aminoacids at about 150°C. Among all these reactions most relevant biological condensation reaction can take place by condensing agents such as polyphosphates and polyphosphate esters which can bring dehydration of peptides from aminoacids on irradiation with ultraviolet or heating.

1.3 THE PREBIOTIC ORIGIN OF POLYPEPTIDES

Polypeptides are proteins which are synthesized by polymerization of amino acids. The experiments conducted by Fox and his colleagues have shown that polymers resembling polypeptides were formed when a mixture of aminoacids are heated or subjected to electric discharge or when they were treated with condensing agent like polyphosphates under
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simulated primitive earth condition. They named it as proteinoids which approach 20,000 daltons. They comprised nearly 18 aminoacids by peptide linkage. They exhibit several other properties characteristics of protein.

Further experiments in the same line of approach indicate that proteinoids may even be formed at low temperature in aqueous environment by a reaction similar to aminoacyl-tRNA in present day protein synthesis. Other hypothesis proposed by Akabori has suggested that polypeptides may have been arised by polymerisation of monomeric units instead of aminoacids.

Any single proteinoid molecule cannot survive in the sea for long time due to unstable peptide bond, since these are thermodynamically not stable and may undergo hydrolytic cleavage in aqueous environment of primordial sea. Hence, improvisation process of aminoacid sequence could have undergone to bring stability to the proteinoid biomolecule. They can be able to survive only for short period. The evolution of enzyme from simple precursors seems probable that enzyme acquired catalytic activity first and followed by substrate specificity as later development in the course of evolution. The first primitive catalyst could have been a simple short polypeptide consisting of $\alpha$-ammonium group, an imidazole ring or a carboxyl group. Further selection and modification of polypeptides may have evolved and formed more potent peptide with more specific catalytic activity. In addition, they were stable and equipped with better survival in the primitive primordial environment. The earliest proteinoids may have been the precursors of enzymes. Several rate enhancing activities have been detected in proteinoids like hydrolysis of esters, decarboxylation of $\alpha$-ketoacids etc. The progress made in the evolution of catalyst was meagre until the availability of long peptide chain which was capable of assuming three dimensional status.

1.4 ORIGIN OF NUCLEIC ACIDS

Several simulated primitive earth experiments were conducted to consolidate information on evolution of nucleic acid molecule. Nucleoside like adenosine and deoxy adenosine have been detected in simulated earth experiment. When nucleosides and polyphosphates are heated or irradiated with UV, nucleotides are formed. Using similar experiment ADP and ATP were generated. Next course of evolution is the evolution of nucleic acids by interlinkages of nucleotides. This was also evidenced by simulated earth experiment in which nucleotides were heated to 60–65°C in the presence of polyphosphoric acid as condensing agent. In the primordial period first polynucleotide contains 2'-5' linkage. However, 3'-5' linkages may have arisen due to availability of specific enzymes which have the ability to enhance 3'-5' linkages of nucleotides.

Since nucleic acids are capable of storing the genetic material and are perfectly suited for the task of replication, it is difficult to image how the first nucleic acid genetic material could be replicated with the participation of protein catalyst. Ambiguity continues as how a protein catalyst could arise without a nucleic acid genome which encodes it. Several classical theories were proposed in 1981 to overcome ambiguity in the first origin of nucleic acids. It was shown that rRNA introns could function as enzyme suggest that very first living molecules might have been an RNA replicase. It can be even suggested that this enzyme catalysed its own replication without help from a protein. This hypothetical RNA might have been behaved functionally as both the genetic material and replication enzyme. In the due course of investigation many new examples of catalytic RNA have been discovered. Hence, catalytic features of RNA suggest that they are molecular fossils. Studies of catalytic RNA threw light on the current views about
the origin of life. The origin of the replicase enzyme can be linked to random prebiotic condensation of mononucleotides which later formed longer and longer polymers. This earliest or first RNA replicase would have been able to use either itself or another nucleic acid as a template for polymerization of nucleic acid precursor found in prebiotic soup.

The first origin of biomolecules like proteins and nucleic acids is under debate. Many are of the view that primitive proteins originated first. The first protocell formed when primitive catalyst was entrapped in gel like matrix or first surrounded by a membrane. During the process some how acquired a primitive metabolism. In contrast it was presumed that first cell functioned in the absence of nucleic acids away from the genetic system, which they acquired in the later stage of evolution. Second approach is that nucleic acid formed first and acquired the genetic information for the evolution of proteins. Third approach is that both the proteins and nucleic acids come together to a closer proximity and evolved to form a first primitive cell. This can be explained on the basis of following hypothesis.

According to Gamow in 1959, there are contrasting differences between aminoacid sequence and nucleic acids, perhaps due to differences in their physiochemical characteristics. The evolution of polypeptide and polnucleotides might have probably occurred simultaneously. It has been postulated that polypeptide with random assembly of aminoacid to possess some catalytic activity.

1.5 ORIGIN OF PROTOBIONT

Protobiont considered as primitive forms of life, might have been originated first in the primeval sea. In order to consolidate this theory, several evolutionary investigations were carried out and proposed some models. They are coacervative model and protenoid model.

1.5.1 Coacervate Model

The first precursors of cells called protobionts arose when membranes formed around macromolecules exhibiting catalytic activity such as proteins. Oparin suggests that their cell phase could have arose from the concentrated primordial broth by the process of coacervation. Thus coacervative droplet that was formed spontaneously when water molecules are organised around electrically charged molecule. Some of which even fused to form complex coacervate. They are able to concentrate organic molecules from their surroundings. Since they contain enzymes which are able to synthesise and degrade many complex molecules such as starch, release maltose into surrounding medium. In the simulated experiment concentrated solution of polypeptides, polysaccharides and even RNA could be induced to form coacervative droplets of different dimensions to which enzymes can be easily incorporated into such droplets and several metabolic activities can be demonstrated. Oparin's coacervative droplets are simply a model and they do not exhibit any self replication process or participate in evolution.

1.5.2 Proteinoids

According to Sydney Fox in 1965, one of the most favourite choice for protobiont is proteinoid. They are protein-like molecules synthesized under invitro condition by polymerization of aminoacids when heated between 130°-160°C. Proteinoids maintain stability between 0-25°C but are unstable at high temperature. In addition, they exhibit weak catalytic properties. It was also found that proteinoids were found to possess an outer membrane boundary resembling plasma-membrane structure which were devoid of lipids.
1.6 ORIGIN OF GENETIC CODE
Several investigators are of the opinion that code on the DNA molecules arose gradually in stages and not formed at once for all. In the evolutionary selection, force for the development of the code must have come from the primitive protein. Primitive polypeptide must have comprised mainly of glycine and other aminoacids which have organised into useful oligopeptide sequence carrying out some primitive functions. Since RNA molecules was postulated as earliest molecules, information in RNA molecules is encoded in the form of triplet codes. But it was a matter of debate on the evolution of this code. Among existing codes those specifying the same aminoacids have common first two bases. However, the third base is different. The existing two base code could code for only 16 types of aminoacids. It was postulated that functions of primitive protein might have produced by the two base genetic code. Due to instability of this doublet codon mode, they could not have stabilized for long time. As a result triplet codon was perhaps formed during the course of natural selection.

According to Francis Crick in 1967, organisation of genetic code was the ultimate result of random interaction over time and space. Finally development of genetic code was stabilized. Any further change in the codon usage during evolution was restricted due to lethal effect that would have inflicted, if any disturbance to the aminoacid sequence.

1.7 BIOCHEMICAL EVOLUTION AND PROKARYOTIC ORIGIN
One of the most simple living organism is mycoplasmas which exhibit many similarities. Mycoplasmas measures 0.3 μm and contain nucleic acid code for about 750 types of proteins. Survival of cell depending on the minimum number of proteins. Both RNA and DNA shows genetic information. Once the primitive cell was evolved, due to competition with others it acquires its own new metabolic engineering status from the surrounding. Due to continuous interaction and rapid changes in the environment mycoplasmas like cells evolved themselves into prokaryotes such as bacteria and blue green algae.

Once the prokaryotes was evolved they were exposed to wide range of shuffled environmental condition. Availability of different organic molecules in the environment compel the bacterial orgaisms to reorient or evolve a new metabolic pathways to effectively utilise the available food material. Thus a new set of enzymes are required for the sustaining of newly evolved pathways. Hence elaborate metabolic pathways might have developed.

In the primitive earth conditions, for the first evolved life, there was little need for elaborate metabolic pathways and were able to sustain on the primordial soup or organic molecules. Availability of natural resources dwindled considerably as the population of cells increased to the greater extent. Due to competition for food even a simple bacteria had to evolve a new line of metabolic pathway in order to utilise more organic food material in the environment. Due to increase in metabolic related enzyme and further cellular adaptation it was suggested that diverse metabolic pathways have invariably evolved in the course of evolution.

1.8 ORIGIN OF EUKARYOTIC SYSTEM
Eukaryotes evolved from large anaerobic prokaryotic cell. Due to increase in demand for energy prokaryotic entities may have gradually transformed into eukaryotic mode of life. Such anaerobes may have engulfed small photosynthetic or heterotrophic prokaryotes, which later became metabolic endosymbionts and are well equipped to furnish glycolytic fermentation and respiratory capacity. It was presumed that endosymbionts may have been the key players for the formation of modern chloroplast and mitochondria.
Establishment of mitochondrial organelle system in eukaryotes provided a significant boost in generating energy which greatly enhanced their survival and adaptation potential besides better equipped to face the environment. They have acquired several modes of differentiation by modifying their own genome. The course of biological evolution is mainly due to the occurrence of non lethal mutation which is the result of chemical or physically induced changes in the base sequence of the genome. According to Sydney Fox efficiency of primitive photosynthesis system was increased by the development of membrane bound lamellae as in the case of cyanobacteria. Emerging oxidised atmosphere stimulated primitive photosynthesizing cell to break up internal photosynthetic membrane in the small sacs. During further course of evolution some cells acquired different version of these membrane sacs. Some of the cells are equipped with well organised chloroplast. Whereas other cell group began to lose their photosynthetic process but retain their electron transport chain and transformed into mitochondria. Normal cells required one set of chromosome. When the genome are subjected for deleterious mutation they were perished in the long term. While others with non lethal and favourable mutation survived besides adapting to new condition.

According to Smith in 1978, nucleus is formed by invagination of the surface membrane which make the boundary surrounding the DNA molecule, which provides compartmentalization to separate genetic material from the rest of the cytoplasm for the formation of nucleus, and circular DNA increases its size to acquire more genetic complexity. Further suggestion reveals that they break up linear DNA into pieces which result into line of division to increase its copy number. Further biochemical evolution evolved lyosomes by enzyme containing vesicles. Besides formation of golgi apparatus and endoplasmic reticulum by mesosomal investigation and its further extension into the cytoplasm as a tubular network.

One of the most significant event in the course of evolution is the appearance of mitochondria and chloroplast in plant cells. The genetic material of these cell organelles especially mitochondrial DNA resembles prokaryotes in possessing circular DNA and reproducing by fission. However, this high energy producing power house organelle favours the eukaryotic system for well adaptation. In the similar fashion chloroplast too share some of the above common features with prokaryotes. Presence of contrasting similarities between eukaryotic photosynthetic organism with cyanobacteria is pertaining to photosynthetic activity. Thus, origin of chloroplast is presumably from ancestral cyanobacteria, which at some stages of evolution was engulfed by eukaryotes. It was evidenced by the agrobacteria like structure of the chloroplast present in the present day moss cells and further evidenced is the occurrence of cyanobacteria in certain present day eukaryotic cells such as cyanophora. Due to continuous organisation of genetic complexity there is considerable increase in gene dosage and well characterized differential gene regulation make the eukaryotic system as well stabilised organism.

1.9 EVOLUTION OF AUTOTROPHIC MODE OF LIFE (PHOTOSYNTHESIS)

The primitive cell in the primeval period were heterotrophic and also exhibited anaerobic mode of life. They used to procure nutrition from the organic soup. In the course of evolution, as the nutrition became scarce due to increasing population of cells, several organisms invariably acquired different mode of nutritional pathway. Some of the organisms synthesized this food material utilising sunlight and green chlorophyll. These are called phototrophs. Origin of chlorophyll in these autotrophs is one of the turning point in acquiring photosynthesis. However, majority of the bacteria derive their energy from inorganic molecules and synthesize their own food material at photo-synthetically.
In the evolutionary adaptation primitive cell modify its genome so that complex array of enzymes can be produced to utilise sun’s energy more efficiently for the synthesis of complex organic food material. In comparison phototrophic bacteria acquired organisational complexity and wide array of enzymes and make the bacteria, a challenging task of reducing carbon dioxide into the complex carbohydrate. The imposing selection pressure makes the phototrophs to reorient themselves into a difficult mode of photosynthetic activity in which water was electron donor in some cells whereas other cells developed a system to release sufficient amount of molecular oxygen into air and water. While some of the cells developed respiratory electron transport chain in which molecular oxygen is the final electron acceptor. In the competition the light sensitive chlorophyll pigments must have evolved to utilise solar energy in photons in competition with earlier process of photophosphorylation of nucleotide to form high energy compounds like ATP.

**SUMMARY**

Primitive earth was formed millions of years back as a hot fire ball devoid of atmosphere. Atoms were formed due to continuous collision between neutrons and protons then joins electron to become atom. Gaseous molecules and water appeared due to torrential rains. Due to several primordial chemical reactions which took place with the help of energy obtained from electric discharge of lightning and sun radiation, primitive earth consisted of wider variety of important bimolecules which acted as building block to construct primitive life on this planet.

The primitive atmosphere of the primeval earth contained mainly carbon dioxide, methane, sulphur dioxide, ammonia without oxygen. Hydrogen was lost due to oxidised component and oxygen appeared much later due to photosynthetic activity. Stanely Miller conducted several experiments under simulated primeval earth conditions. He had synthesized organic compounds under invitro conditions by mixing methane, hydrogen and ammonia in presence of water. In addition several intermediate products were produced in invitro experiments like hydrogen cyanide, aldehyde etc. These intermediate were important molecules in the formation of aminoacids and nucleic acids.

Several chemical organisation resulted in complex biomolecules by possible covalent linkages. The building blocks forms more complex molecules such as polysaccharides, polypeptides and polynucleotides. Several biological condensation reactions took place by condensing agents such as polyphosphates and polyphosphate esters which can bring dehydration of peptides from aminoacids. Several simulated experiments were conducted to evidence origin of polypeptides. The experiments include the heating of mixture of aminoacids or were exposed to electric discharge or to condensing agent like polyphosphates and obtained polymer resembling polypeptides known as proteinoids. Due to unstable nature of proteinoids molecule they undergo hydrolytic cleavage. Hence, improvisation of proteinoids aminoacids could have undergone to bring stability to the proteinoid biomolecule. Then enzymes were evolved from simple precursors. It seems probably that enzyme acquired catalytic activity first and followed by substrate specificity as later development in the course of evolution.

Additional simulated experiments were conducted to consolidate evidence on evolution of nucleic acid molecule. In the invitro experiments nucleosides and polyphosphates were heated irradiated with UV as a result of which nucleotides were formed. In the next course of evolution is the evolution of nucleic acid by interlinkages of nucleotides. There was ambiguity on how the first nucleic acid was able to replicate and how a protein catalyst could arise
without a nucleic acid genome which encode it. It was evidenced that first living molecules might have been a RNA replicase and are responsible for its own replication. This RNA behaved as genetic material as well as replication enzyme. Origin of replicase enzyme can be linked to the formation of long polymers. Several evidences proclaim that primitive proteins originated first and the first cell functioned in the absence of nucleic acids. Another view consolidates that nucleic acid formed first and acquired the genetic information for the evolution of proteins. Another approach claims that both protein and nucleic acid come to closer proximity and evolved to form primitive cell.

Protobiont was considered as primitive form of life and acts as precursors of cells. They could have arose from the concentrated primordial broth by the process of coacervation. The complex coacervates are formed in the later stage which acquired enzymes capable of degrading several molecules. Investigations are of the opinion that code on the DNA molecules arose gradually. Codes must have come from the primitive protein. First triplet code might have been in RNA molecule since it formed first. Unstable doublet codon perished, immediately as a result triplet codons were formed as a natural selection.

After the origin of prokaryotes they were exposed to wide range environmental conditions. As a result bacteria were able to acquire new metabolic pathways and reoriented themselves to utilise new food material in the environment. Hence an elaborate metabolic pathway might have developed. During the course of evolution there was a serial transformation from the earliest heterotropic mode to autotrophic mode of life. Some of the organisms started synthesizing their food material using sunlight and green pigments. These are called phototrophs. Chlorophyll origin was one of the turning point in acquiring photosynthesis.

Eukaryotes evolved from large anaerobic prokaryotic cell. As the requirement for energy demand increases considerably, prokaryotes might have transformed into eukaryotic mode of life. Due to engulfing of small prokaryotes the eukaryotes were able to acquire vital cell organelles during the course of evolution. Acquiring mitochondrial organelle by eukaryotes provides a significant boost in generating energy. One of the most significant event in the course of evolution is the appearance of mitochondria and chloroplast in plant cells. Organisation of complexity of the genome, differential gene regulation and stability of biomolecules are some of the highlights among eukaryotes as they as dominating all life forms on this planet.

**QUESTIONS**

1. Discuss the process of evolution of organic biomolecules.
2. Explain different concepts, and evidences in favour of molecular basis of life.

**SHORT QUESTIONS**

1. Coacervative mechanism
2. Miller’s experiment
3. Organisation of prokaryotic cell
4. Evolution of eukaryotic cell