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Origins and early evolution of life

Tairo Oshima

Department of Life Science, Tokyo Institute of Technology,
Nagatsuta, Yokohama 227, Japan.

The fundamental properties of life were determined when life first arose and evolved into the common ancestor to the contemporary organisms. Thus in order to understand the chemical basis of life, it seems essential to solve the prebiotic process and early history of life. Unfortunately only little has been settled so far. Prebiotic organic synthesis is still one of the important, unsolved problems. The primitive atmosphere would be more oxidized one than methane-ammonia-hydrogen used in the classical Urey-Miller experiment. We found that proton irradiation, the most abundant energy source in the vicinity around the Earth, is effective for abiotic organic synthesis from mildly oxidized gas mixtures.

One of the recent progress in the field of Molecular Evolution is construction of a molecular phylogenetic tree with the root. According to the tree, the closest organisms to the primitive life are hyperthermophiles. The results suggest that the common ancestor, from which three kingdoms of the contemporary organisms diverged, was a hyperthermophile and the proteins at the beginning were extremely robust to heat.

1 Origins of Life

Origins of life is an attractive and important subject. But little has been settled so far. It is generally believed that life originated spontaneously in the process of chemical evolution in the primitive ocean about 4 billion years ago. Many theories have been proposed to explain how life started from the primordial soup, but every theory contains controversial points.

Life can be regarded as an evolving chemical machine consisting of two basic functions; replication and metabolism. von Neumann pointed out first time that a self-replicating machine should consist of two essential components; a program or software and a machine to operate the program or hardware. Nucleic acid (RNA or DNA) is software of life, and protein is hardware of life.

In the contemporary organisms, replication and energy metabolism are linked intricately, but they are logically separable. Thus origin of life is another chicken-and-egg problem. Most of the theories can be classified into three groups;

theories of nucleic acid-based primitive life, protein-based life, or theories based on the rare event that the two components were assembled together by an accident. Recent popular theory is RNA origin of life.

RNA has long been thought to be only a carrier of genetic information, but the discovery of catalytic functions of RNA (ribozymes) (1), that is, some RNA molecules act as catalyses, changed our image of roles of RNA in chemical evolution. A possibility has been proposed that life started from a primitive cell based on RNA(2,3).

The concept of RNA world is popular today, but I wish to emphasize the fact that RNA, in comparison with polypeptides, is rather difficult compound to synthesize abiotically under primitive Earth conditions. Firstly, many simulated primitive Earth experiments indicated that nucleic acid bases and ribose, the components of RNA, are produced only in a small quantity compared to amino acids which are major products in these experiments(4).

Secondly, ribose contains so many reactive hydroxy groups and linkage specificity causes difficult problems. Yield of nucleotides with correct structures is very low if bases, ribose, and phosphate are mixed and heated in the absence of enzymes. Only a small amount of nucleosides or nucleotides with proper linkage specificities was produced in such primitive Earth experiments. In contrast, amino acids such as glycine, alanine, valine, phenylalanine, and so on contain no reactive group in their side chains, thus protein-like polymers with alpha peptide bonds are major products when amino acids react in the presence of suitable condensing agents.

Especially if the primitive ocean was hot due to the green house effect of a large amount of carbon dioxide in the primitive atmosphere, RNA molecules could not last for a long time because chemical linkages in RNA are more heat labile than a peptide bond is, and phosphodiester bonds could be hydrolyzed within a short period.

Thus I wish to stress that RNA-world is not the unequivocal answer for the origin of life. Likewise almost every aspect of the study of origins of life has not been completely solved yet. In this presentation, I will report two recent results of our studies on origins of life.

2 Prebiological Synthesis of Organic Compounds

Origins of life can be experimentally studied by two different types of approach; one is to trace the history from the beginning, and the other is to trace back from the present to the past.

A large number of experiments have been performed since the famous Miller-Urey experiment in the early 50's(5) to prove how molecules of biological interest such as amino acids were formed and accumulated on the primitive Earth. It has been reported that almost every member of basic molecules of life such as amino acids, nucleic acid bases, sugars, organic acids, hydrocarbons, and porphyrins can be produced from a mixture of methane, ammonia, hydrogen, and water under the simulated primordial Earth conditions. Even the formations of polypeptides and cell-like structures have been reported by many investigators.

However, unsolved problems still exist in this very first step of chemical evolution. Recent geochemical studies suggested that the primitive atmosphere would not have been strongly reduced gas mixture, but more oxidized one due to high heat produced by high-velocity impacts of planetesimals onto a growing Earth(6). It has been shown that only a small amount of amino acids was formed in the electric discharge experiments using oxidized or mildly oxidized gas mixture(4).

In collaboration with Dr. K. Kobayashi of Yokohama National University, we studied the effects of proton irradiation on the abiotic organic synthesis using a mildly oxidized mixture consisting of carbon monoxide, nitrogen, and water. High energy protons are the major components of cosmic rays and solar flare particles.

The mildly oxidized gas mixture was irradiated with high energy protons (2.8-4.0 MeV, 10-600 nA) generated from a Van deGraff accelerator in our University for 2 hours at 25^{o}C.

After the irradiation, the products were analyzed for amino acid or nucleic acid base. For amino acid analysis, the products were treated with 6N HCl at 105^{o}C for one day. Amino acids were identified using LI-MS (liquid ionization mass spectrometry). For nucleic acid bases, the products were separated with a reversed-phase column chromatography and then a cation exchange HPLC.

Several amino acids were identified and quantitatively analyzed(7,8). In an experiment, about 6 micro moles of glycine were formed with G-value of 0.017 (a number of glycine molecules formed with energy deposit of 100 eV). Other amino acids identified were alanine, aspartic acid, serine, threonine, glutamic acid, alpha-aminobutyric acid, and beta-alanine.

Among bases, uracil and dihydrouracil were identified in the products by proton

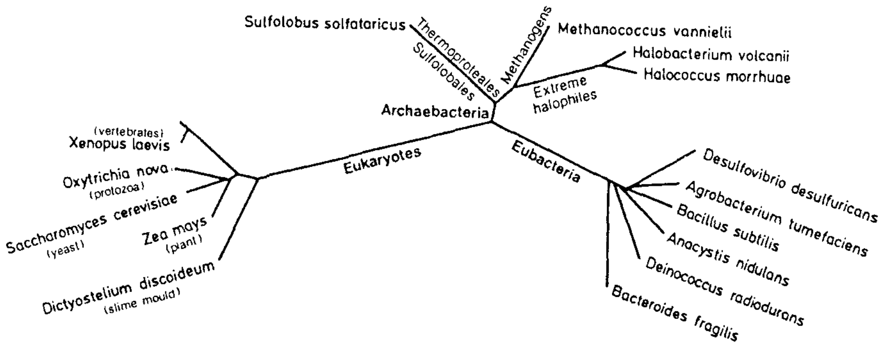


Fig. 1. A phylogenetic tree drown from rRNA sequences (from Ref. 11).

irradiation of a $CO-N_2-H_2O$ mixture(9). Imidazole was also identified by LI-MS.

The present study indicates that amino acids and nucleic acid bases could have been abiotically synthesized on the primitive Earth even if the atmosphere was not a strongly reduced one. The results also suggest that biologically related compounds present in meteorites could be formed by proton irradiations since protons are the major energy source, and carbon monoxide, nitrogen, and water molecules exist commonly in the inner region of the solar system.

3 Phylogenetic Tree with the Root

The second approach is to retrace biochemical evolution and to speculate on the biochemistry of the primitive life. In this field, one of the latest topics is discovery of archaebacteria. A kingdom called Archaebacteria was proposed as the third group of life by Woese and Fox(10) based on sequence analysis of a ribosomal RNA. They proposed that life can be divided into three major domains; eubacteria, archaebacteria, and eukaryotes (or Bacteria, Archaea, and Eukarya).

Fig. 1 shows a molecular phylogenetic tree calculated from ribosomal RNA sequences by Woese and Olsen(11). In this figure, the length of a line indicates relatedness between two organisms. As suggested from the tree, biochemistry of archaebacteria is clearly distinguished from those of eubacteria and eukaryotes. However, this tree has no root. The position of the common ancestor is not clear in this molecular tree.

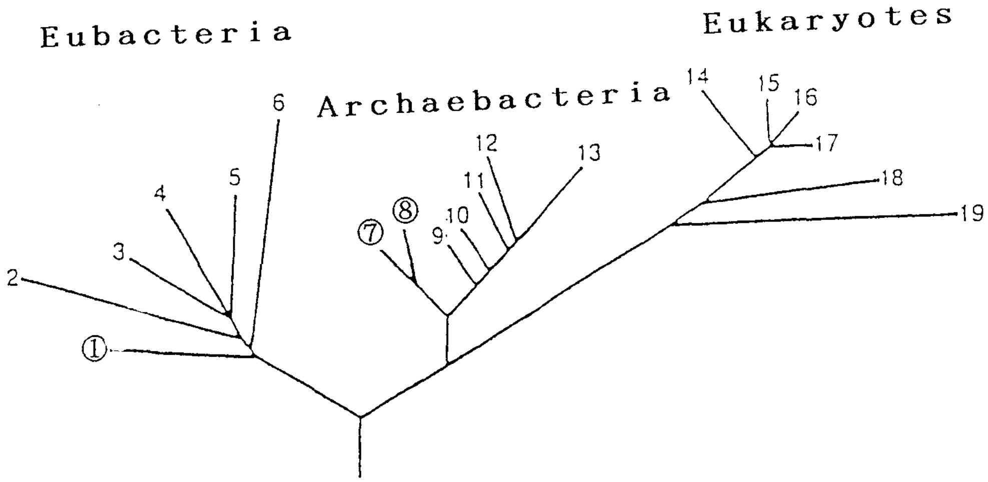
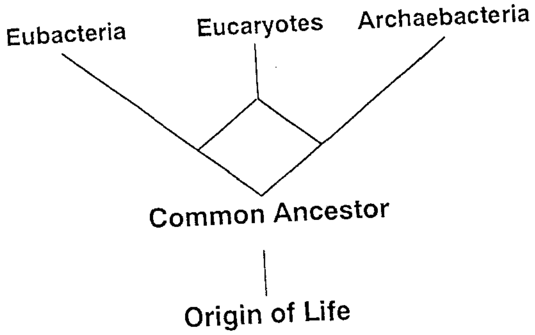


Fig. 2. A rooted tree. Hyperthermophiles are the closest organisms to the common ancestor. Organisms indicated by circles in the figure are hyperthermophiles.

Quite recently the root of the tree was placed by two research groups independently based on sequence homology of twin proteins such as ATPase alpha and beta subunits, and elongation factors Tu and G(12,13). Fig. 2 shows the rooted tree. The root was placed between eubacteria and archaebacteria. The tree supports the archaebacterial origin of eukaryote cytoplasm as suggested from sequence homology of ATPases(14).



Mitochondria and chloroplast of eukaryotes are derived from invasions of eubacteria into the primitive eukaryote cells. Similar endosymbiotic invasions and gene transfer from archaebacteria into the primitive eukaryote cells could have taken place repeatedly in the early history of life as suggested by Hartman(15). Thus contemporary eukaryote cells can be regarded as hybrids or mosaic of eubac teria and archaebacteria (Fig. 3). For instance,

Fig. 3. A phylogenetic tree of new dichotomy.

membrane bound ATP synthesizing ATPase in eukaryote mitochondria might come from the eubacterial ATPase, and ATPases on vacuola and lysosome membranes of eukaryote cytoplasm might originated from the archaebacterial enzyme(14).

In a sense, the primitive organism began to diverge into two groups, eubacterial and archaebacterial lineages. We proposed to name the common ancestor as the "commonote" (Yamagishi and Oshima, submitted). The most important event of the history of life on our planet could be the divergence of the commonote into these two lineages. Eukaryote cells were assembled by mixing these two bacterial cell components.

The biochemistry of the commonote can be speculated from the comparison of biochemistry of eubacteria and archaebacteria. If a common biochemistry is found, this would have been the biochemistry of the commonote. If biochemical properties of eubacteria and archaebacteria are different, then the simpler or less efficient one would have been shared with the commonote.

Proteins at the Beginning

The rooted tree indicates that the deepest branch is either eubacterial (Thermotoga) or archaebacterial (Acidianus, Desulfurolobus or Sulfolobus) hyperthermophiles (Fig. 2). Hyperthermophiles are organisms capable of growing at 90^{o}C or higher temperature(16). It is most likely that the commonote was a hyperthermophile and lived in hot environment.

The growth temperature of these organisms is in a range of 90-110^{o}C. Thus the growth temperature of the commonote might have been around 100^{o}C. This consideration is in good agreement with the latest hypothesis on the surface temperature of the primitive Earth; the major atmospheric component was carbon dioxide and the surface temperature was much higher than that today due to green house effect.

Proteins from the hyperthermophiles or extreme thermophiles are unusually stable to heat. In some cases, enzyme activity remained after heating at 100^{o}C. These findings suggest that proteins in the commonote were extremely resistant to heat.

It has been shown that only a small fraction of hyperthermophiles inhabiting in hydrothermal environments has been isolated and characterized in laboratories so far(17). So that it seems to be necessary to isolate as many hyperthermophiles as possible from hot springs, hydrothermal vents, or under the sea volcanoes in order to identify the most primitive life on this planet.

So far many unusually stable enzyme proteins from thermophiles have been extensively studied(18,19). However, the secret for the unusual stability has not been uncovered fully. No remarkable change has been found in their primary sequences and three dimensional structures. It is generally accepted that only subtle changes in amino acid sequence and in side chain conformation are responsible for the increased stability of the thermophile proteins.

For instance, increment of hydrophobic interaction at the subunit-subunit interface of a thermophile enzyme confers at least a part of the unusual stability to heat(20). Catalytic functions remained generally unchanged throughout the adaptation to high heat. It seems that the change in structure which is necessary for making the thermophile protein heat stable, did not affect the function. Catalytic properties of thermophile enzymes are often similar to those of the mesophile counterparts. It can be speculated that proteins in the commonote might consist of the same 20 amino acids as the contemporary proteins and their structures were similar to those of the contemporary enzymes, but extremely stable to heat.

Proteins known to most of biochemists today could be deteriorated mutants of the stable primitive proteins. Many mutations which did not change the catalytic functions, but are harmful for the stability at high temperature extreme, might be accumulated during 4 billion years history of evolution. Such mutations were neutral and could be fixed accidentally after most of carbon dioxide in the primordial atmosphere had disappeared and the surface temperature of the Earth had been cooled down to the present level.

Structure-stability relationship of RNA molecules of hyperthermophiles would be another interesting subject in connection with the primitive RNA or RNA-origin of life. Chemical structures of tRNAs from hyperthermophiles have been investigated and it has been reported that the modifications are responsible for the stability of the molecules at the high temperature extremes(21). No ribozyme activity has been reported for hyperthermophiles.

References

1 T.R.Cech, A.G.Zaug, and P.J.Grabowski, Cell, 27 (1981) 487.

2 N.R.Pace and T.L.Marsh, Origins of Life, 16 (1985) 97.

3 W.Gilbert, Nature, 319 (1986) 618.

4 G.Schlesinger and S.L.Miller, J. Mol. Evol.,

19 (1983) 376.

5 S.L.Miller, Science 117 (1953) 528.

6 J.Kasting, Origins of Life, 20 (1990) 199.

7 K.Kobayashi, M.Tsuchiya, T.Oshima, and H.Yanagawa, Origins of Life, 20 (1990) 99.

8 K.Kobayashi, T.Kaneko, T.Kobayashi, L.Hui, M.Tsuchiya, T.Saito, and T.Oshima, Anal. Sci., 7 (1991) 921.

9 K.Kobayashi, R.Hasegawa, K.Yanagi, T.Kobayashi, T.Kaneko, M.Tsuchiya, and T.Oshima, Anal. Sci., 7 (1991) 925.

10 C.R.Woese and G.E.Fox, Proc. Natl. Acad. Sci. USA., 74 (1977) 5088.

11 C.R.Woese and G.J.Olsen, System. Appl. Microbiol., 7 (1986) 161.

12 N.Iwabe, K.Kuma, M.Hasegawa, S.Osawa, and T.Miyata, Proc. Natl. Acad. Sci. USA., 86 (1989) 9355.

13 T.P.Linkila and J.P.Gogarten, Trends Biochem. Sci., 16 (1991) 287.

14 J.P.Gogarten, H.Kibak, P.Dittrich, L.Taiz, E.J.Bowman, B.J.Bowman, M.F.Manolson, R.J.Poole, T.Date, T.Oshima, J.Konishi, K.Denda, and M.Yoshida, Proc. Natl. Acad. Sci. USA., 86 (1989) 6661.

15 H.Hartman, Speculations in Science and Technology, 7 (1984) 77

16 K.O.Stetter, in Thermophiles: General, Molecular, and Applied Microbiology (T.D.Brock, ed.), pp.39-74, John Willy & Sons, New York (1986)

17 D.M.Ward, R.Weller, M.M.Bateson, Nature, 345 (1990) 63.

18 T.Oshima, in "Protein Engineering: Application in Science, Medicine, and Industry" (R.Sarma and M.Inouye, eds.) pp.81-93, Academic Press, New York (1986)

19 H.R.Costantino, S.H.Brown, and R.M.Kelly, J. Bacteriol., 172 (1990) 3654.

20 J.I.Harris and M.Waters, The Enzymes, Vol. 4 (P.D.Boyer, ed.) pp.1-49, Academic Press, New York (1976)

21 C.G.Edmonds, P.F.Crain, R.Gupta, T.Hashizume, C.H.Hocart, J.A.Kowalak, S.C.Pomerantz, K.O.Stetter, and J.A.McClosky, J. Bacteriol., 173 (1991) 3138.

Tairo Oshima was born in Tokyo on Feburary 13, 1935. He studied chemistry at the University of Tokyo, and biochemistry at the Graduate School of the University of Tokyo. He was an assistant professor of the University of Tokyo, and then a division head at Mitsubishi-Kasei Institute of Life Sciences. Since 1983, he is a professor of biochemistry at Tokyo Institute of Technology, Nagatsuta. His research interests have been in biochemistry, molecular biology, and molecular evolution of thermophilic bacteria and archaebacteria, space biology, and origins of life.