International Workshop

"Reconstructing the Phenomenon of Life –To Retrace the Emergence of Life –"

SCOPE

The questions about what kind of pre-life form had led to the formation of a cell prototype more than 4 billion years ago, and how these developed into primitive cells are the central theme in the study of the Origin of Life. Trying to reconstruct the phenomenon of life by creating a simple living cell is important because it is synonymous with retracing the process of the emergence of life. By this approach, we may practically understand the hierarchical dynamics in life and may find a universal principle that is common to life. In this workshop there will be several important keynote speakers and young researchers who will present their latest findings from tackling these questions with constructive approaches. This workshop also aims to encourage Origin of Life researchers for many generations.

Date & Venue

Date: May 31, 2017

Venue: ELSI-1 Main Hall, Earth-Life Science Institute (ELSI), Tokyo Institute of Technology, Ookayama, Meguroku, Tokyo, Japan (http://www.elsi.jp/en/access.html)

Keynote Speakers

Jack Szostak (Howard Hughes Medical Institute, Harvard University)

David Deamer (University of California, Santa Cruz)

Irene Chen (University of California, Santa Barbara)

Sudha Rajamani (Indian Institute of Science Education and Research, Pune)

Program

08:30 –09:00	Reception
09:00 –09:05	"Opening Remark" **Kei Hirose** (*ELSI Director*)
09:05 –09:10	"Introduction" **Yutetsu Kuruma** (*Organizer; ELSI*)
09:10 –09:50	"Hydrothermal vents or hydrothermal fields: Where can life begin?" **David Deamer** (*Univ. of California*)
09:50 –10:30	"The Origin of Cellular Life " **Jack Szostak** (*Harvard Univ.*)
10:30 –10:50	*Coffee Break*
10:50 –11:20	"Informational molecules of a pre-RNA World" **Sudha Rajamani** (*IISER, PUNE*)
11:20 –11:50	"Fitness landscapes in the RNA World" **Irene Chen** (*Univ. of California*)
11:50 –13:20	*Lunch at ELSI-2 Lounge (& Photo Shoot)*
13:20 –13:50	"Evolutional history of Hadean surface environment and three step model for the emergence of first life" **Shigenori Maruyama** (*ELSI*)
13:50 –14:20	"Radiolytic Synthesis of RNA Precursors" **Albert Fahrenbach** (*ELSI; Harvard Univ.*)
14:20 –14:50	"Strategy for origin of life research using chemical artificial cells" **Kensuke Kurihara** (*Institute for Molecular Science*)
14:50 –15:10	*Coffee Break*
15:10 –15:40	"Concentrating cell extract to reveal relation between macromolecular concentration and biological activity" **Kei Fujiwara** (*Keio Univ.*)
15:40 –16:10	"Evolution of a liposomal artificial cell through repetitive destruction-reproduction cycles" **Takeshi Sunami** (*Osaka Univ*.)
16:10 –16:20	*Coffee Break*
16:20 –17:20	**Discussion with Invited Speakers**
17:20 –17:25	"Closing" Yutetsu Kuruma

Hydrothermal vents or hydrothermal fields: Where can life begin?

David Deamer

Department of Biomolecular Engineering, University of California

Because salty seawater dominates the Earth's surface today, it is natural to think that life began in the ocean, perhaps in hydrothermal vents (1 - 4). However, from a biophysical perspective there are significant limitations to this conjecture, particularly in terms of polymerization, thermodynamics and self-assembly of membranous compartments. An alternative site is hydrothermal fresh water conditions associated with volcanic land masses. During field work in volcanic regions of Kamchatka (5), Iceland and Hawaii, we found abundant fresh water pools that undergo cycles of hydration (precipitation) and dehydration (evaporation). We established a laboratory simulation of such cycles to test whether important polymers like RNA could be synthesized. In a typical experiment, solutions of mononucleotides were mixed with lipids that can assemble into membranous structures. When the mixtures were exposed to multiple wet-dry cycles we found that RNA-like polymers from 10 to over 100 nucleotides in length had been synthesized, confirming that the chemical energy made available by cycles of hydration and dehydration in a hydrothermal field is sufficient to drive synthesis of ester bonds (6, 7). Furthermore, in the final hydration phase the polymers become encapsulated in lipid vesicles to produce protocells. It is unlikely that similar polymerization reactions can take place in hydrothermal vent conditions because there is no plausible energy source available to drive condensation reactions (8), and the high concentrations of salt and divalent cations (Ca++ and Mg++) inhibit self assembly of membranes and encapsulation processes (9). We are now testing a variety of protocells to determine whether certain polymer combinations can promote their survival in multiple cycles. If the experiments are successful, we will conclude that selection and evolution of robust protocells composed of encapsulated catalysts (10) could be the first step toward the origin of life. Only afterwards was primitive cellular life transported into marine conditions and adapted to the salt concentrations of sea water.

1. Martin W, Russell MJ (2007) On the origin of biochemistry at an alkaline hydrothermal vent. *Phil Trans R Soc Lond B* 362:1887-1925

2. Russell MJ, Hall AJ, Martin W (2010) Serpentinization as a source of energy at the origin of life. *Geobiology* 8:355-371
3. Lane N, Martin WF (2012) The origin of membrane bioenergetics. *Cell* 151:1406-1416
4. Herschy B, Whicher A, Camprubi E, Watson C, Dartnell L, Ward J, Evans JRG, Lane N (2014) An Origin-of-Life Reactor to Simulate Alkaline Hydrothermal Vents. *J Mol Evol* 79:213–227.
5. Deamer D, Singaram S, Rajamani S, Kompanichenko V, Guggenheim S. (2006). Self-assembly processes in the prebiotic environment. *Philos Trans R Soc Lond B* 361, 1809-18.
6 Rajamani S, Vlassov A, Benner S, Coombs A, Olasagasti, Deamer D. (2008) *Orig Life Evol Biosph.* 38, 57-74.
7. De Guzman V, Shenasa H, Vercoutere W, Deamer D. (2014) Generation of oligonucleotides under hydrothermal conditions by non-enzymatic polymerization. *J Mol Evol* 78, 251-262
8. Jackson B. (2016). Natural pH gradients in hydrothermal alkali vents were unlikely to have played a role in the origin of life. *J Mol Evol* 83: 1-11.
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10. Adamala KP, Engelhart AE, Szostak JW (2016) Collaboration between primitive cell membranes and soluble catalysts. *Nat Commun* | DOI: 10.1038/ncomms11041.

The Origin of Cellular Life

Jack Szostak

Howard Hughes Medical Institute, Harvard University

The complexity of modern biological life has long made it difficult to understand how life could emerge spontaneously from the chemistry of the early earth. We are attempting to synthesize simple artificial cells in order to discover plausible pathways for the transition from chemistry to biology. Very primitive cells may have consisted of a self-replicating nucleic acid genome, encapsulated by a self-replicating cell membrane. A chemically rich environment that provided the building blocks of membranes, nucleic acids and peptides, along with sources of chemical energy, could have led to the emergence of replicating, evolving cells. However, no process for the replication of a nucleic acid genome, independent of evolved enzymatic machinery, has yet been described. I will discuss our recent progress towards the realization of an efficient and accurate system for the chemical replication of RNA.

Informational molecules of a pre-RNA World

Sudha Rajamani

Indian Institute of Science Education and Research, Pune

The origin of informational molecules is thought to have been fundamental to the origin of life on the early Earth. An important molecule in this context is the RNA, which has been proposed to play a crucial role in the transition from chemistry to biology. However, the nonenzymatic oligomerization of RNA molecules has been problematic, to say the least. This is because of the inherent instability of the glycosidic bond in extant nucleotides. These are especially prone to hydrolysis under the harsh volcanic geothermal conditions of the early Earth, possibly indicating a lower fitness for modern nucleobases under prebiotic conditions. This observation is also corroborated by studies that attempted to synthesize nucleosides with extant bases. On the other hand, "alternate" bases have been shown to result in nucleosides in higher yields, suggesting a viable and prebiotically relevant solution to the longstanding "nucleoside problem". Importantly, we successfully demonstrated the synthesis of a pre-RNA World nucleotide using ribose 5'-monophosphate (rMP) and barbituric acid (BA) under prebiotically relevant conditions. Polymerization of this BA-nucleotide was also observed when subjected to dehydration-rehydration cycles at low pH and high temperatures. The resultant RNA-like oligomers had intact bases in contrast to what was observed in parallel reactions that were carried out with canonical nucleotides whose glycosidic bond were mostly hydrolyzed. Furthermore, incorporation of BA directly onto preformed sugar-phosphate backbones was also observed when rMP oligomers were subjected to heating with BA. These observations provide preliminary evidence that "alternate" bases would have indeed gotten incorporated into early polymers that could have predated the molecules of an RNA-World. Importantly, it also highlights the possibility that other relevant heterocycles in the prebiotic soup could have been sampled in a similar manner that would have resulted in primitive informational polymers of putative pre-RNA World(s).

Fitness landscapes in the RNA World

Irene Chen

University of California, Santa Barbara

Life probably progressed through a primitive form based on RNA, in which RNA acted as both a genetic material and a catalyst for biochemistry. Understanding the evolution of RNA is therefore central to understanding the origin of life. Evolution can be thought of as a random walk through the space of all possible sequences. The function of fitness in sequence space is known as the 'fitness landscape.' If the fitness landscape were known, evolution could be accurately modeled as diffusion on the landscape with a tendency to drift upward due to natural selection. The fitness landscape is difficult to interrogate due to the vast size of sequence space. However, with high-throughput sequencing, we are able to map fitness landscapes for short but functional sequences of RNA, thus gaining a comprehensive 'birds-eye view' of the fitness landscape and discovering viable evolutionary pathways. One implication of our findings so far is that the ability of natural selection to optimize function across sequence space would be frustrated by the topology of the landscape, which consists of isolated islands of functional sequences. We are also studying the probability distribution of fitness and catalytic activity by analyzing the *in vitro* evolution of longer RNA sequences. This analysis reveals a log-normal distribution of rate constants, suggesting a mechanism for the emergence of function as the multiplicative result of many independent contributions. I will discuss implications of this research on understanding the probability of emergence of functional RNA and the role of chance and the repeatability of evolution in the RNA World.

Evolutional history of Hadean surface environment and three step model for the emergence of first life

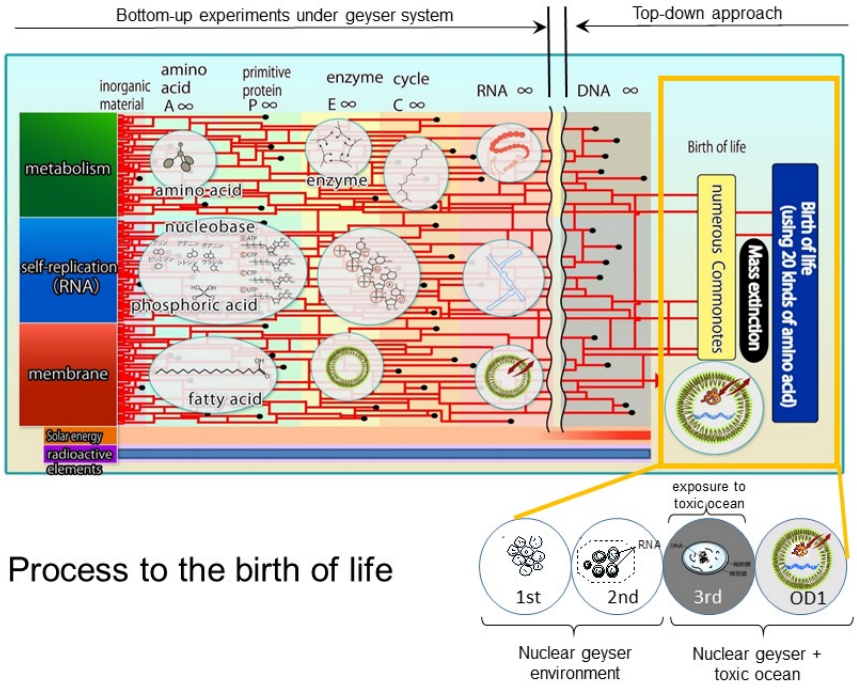
Shigenori Maruyama, Toshikazu Ebisuzaki, and Hadean Bioscience project team

The mystery of the origin of life cannot be solved only by biologist. This topic is solved only when multidisciplinary studies are conducted by all of scientists including astronomer, geochemist, chemist, and geologists. Our research group, mainly geologist, has revealed whole Earth history as cradle of life by two strategies; 4.6-billion-year Historical Approach and Singularity Analytic Approach. Through these two approaches, we found out what necessary condition for the emergence of life was, and how the first life was, which provided 3 step model for the emergence of first life. This model explains that life was born through 3 steps, first stage of proto-life, second stage of proto-life, and third stage of proto-life which is prokaryote. Details are as follows.

The first primordial life was born at natural nuclear geyser and living as extracellular symbiont. They should have survived by making symbiont as primitive ecosystem. Probably numerous numbers of small organelle (similar to present virus) existed. To enable them to survive, energy must have been supplied. Nuclear geyser played this important role. On Hadean Earth surface environment, Sun energy cannot be used and surface environment was too dangerous without geomagnetic field. Instead, nuclear geyser supplies necessary energy continuously for proto-life.

Due to spout of geyser, first proto-life was tossed out of geyser to die due to lack of sufficient energy. They were left on the surface as tahr. After primordial atmosphere became thin enough to pass sun light, life can utilize sun energy on the surface of the Earth. Such life is second proto-life, which obtained new function to utilize solar energy by using principle of semi-conductor. Second primordial life was still extracellular symbiont.

Primordial ocean was too toxic with high acidity, high salinity, and heavy metals. Therefore, second proto-life experienced mass extinction due to influx of toxic ocean as a result of operation of plate tectonics. In spite of repeated mass extinction, some life could survive under strong outer force. Such life became the first life, in other words, first prokaryote on the Earth.



Process to the birth of life

Radiolytic Synthesis of RNA Precursors

Albert C. Fahrenbach [1,2]

[1]Howard Hughes Medical Institute, Harvard University; [2]Earth Life Science Institute, Tokyo Institute of Technology

The RNA world hypothesis has long been challenged by the prebiotic synthesis of the canonical A/U/G/C monomers. Using the potentially prebiotic synthesis of the pyrimidine nucleotides C and U reported by Sutherland and coworkers in 2009 as a blueprint, I demonstrate how gamma-radiolysis of dilute solutions of hydrogen cyanide can lead to the production of many of the key intermediates in that pathway. An additional route for the production of imidazoles was also discovered – a class of compounds crucial for enabling efficient template-directed synthesis of RNA oligomers. Details of these radiolytically driven mechanisms will be discussed.

Strategy for origin of life research using chemical artificial cells

Kensuke Kurihara[1,2]

[1]Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences; [2]Institute for Molecular Science, National Institutes of Natural Sciences

There are various theories concerning life's origin, such as the RNA world and protein world hypotheses. However, a more recent hypothesis, the lipid world scenario, states that compartments capable of self-reproduction gradually acquired the ability to replicate information [1]. A problem with attempting to mimic this scenario experimentally is that once the lipid boundary is formed in water, it is difficult for the molecular system that constitutes the metabolic system or the information replicating system to penetrate or stay in the compartment membrane. We propose that this problem can be solved by creating a hydrophobic oil droplet before using a chemical approach to make the compartment.

We constructed an oil droplet system consisting mainly of octylaniline, which self-propagates spontaneously [2]. Catalytic aldehyde reacted with the octylaniline in the oil droplet and obtained hydrophobicity. The oil droplet incorporated more octylaniline (feed), grew, and divided. When another aldehyde (i.e., membrane precursor) was added to this self-reproducing oil droplet system, it reacted with the internal octylaniline to rapidly generate giant vesicles explosively by the catalyst.

We aim to introduce another reaction system into the vesicular membrane by using the oil droplet-vesicle transformation system. In this workshop, we will talk about fusing membrane-synthesizing oil droplets with peptide-synthesizing oil droplets to encapsulate a peptide synthesis system to produce primitive proteins.

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Concentrating cell extract to reveal relation between macromolecular concentration and biological activity

Kei Fujiwara

Department of Biosciences and Informatics, Keio University

Inner media of living cells are crowded with a very high density of macromolecules at ~30 w/v%. Because of its great influence on chemical reactions, the macromolecular crowding condition has been considered as an important environment for the origin of life. The importance of the macromolecular crowding brings a simple question: "How dense were macromolecules at the origin of life?" To address the question, we have analyzed the effect of concentration on intracellular components extracted from living cells by constitutive approaches [1-3].

Although cell extract prepared from E. coli is able to synthesize proteins from DNA, its efficiency decreased both at lower and higher concentrations [1]. Protein synthesis activity was lost by dilution of cell extract, but recovered by concentrating by water evaporation. Also, loss of protein synthesis in dense cell extract was recovered by dilution. A computational simulation using a simple model for concentration effects on transcription and translation recapitulated the cause of its poor efficiency under dilution, but failed to explain the low activity at the high concentration. We found macromolecular diffusion in cell extract remarkably slowed down at near intracellular concentration, which suggests slow diffusion inhibits protein synthesis. These behaviors were also observed when cell extracts were encapsulated inside lipid membranes [2].

Finally, we demonstrated that the loss of protein synthesis in diluted cell extracts was able to be recovered by concentrating them inside liposomes. Because it seems to rarely happen that macromolecules spontaneously concentrated to the present intracellular levels at the origin of life, our result provides a fascinating scenario that diluted components encapsulated in lipids membranes were concentrated to enough levels for efficient activities

of biological systems by environmental events.

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2. K. Fujiwara, M. Yanagisawa. ***ACS Synthetic Biology*** 3 (12), pp 870-874 (2014)
3. K. Fujiwara, M. Yanagisawa, S.M. Nomura. ***BIOPHYSICS***, 10 (0), pp. 43-48 (2014)

Evolution of a liposomal artificial cell through repetitive destruction-reproduction cycles

Takeshi Sunami

Institute for Academic Initiatives, Osaka University

The ability to evolve is an essential feature of life. To reconstruct this characteristic using well-defined materials, I designed a primitive cell model that can proliferate through successive cultures. For the cell proliferation, I adopted three steps cycle: the formation of cells, gene replication in the cells, and the destruction of the cells. In the repetitive cycles, daughter cells are produced from supplied nutrients and the genes leaked from their parent cells. Giant liposomes were used as cell-like microcontainers and multiplied through the addition of lipids and repetition of desiccation-hydration cycles. RNA was used as the genetic material and was repetitively replicated by Qβ replicase. My primitive cell model was successively cultured through 52 rounds of desiccation-hydration cycles. After these successive cultures, the inherited RNA was replicated more quickly relative to the native RNA. Furthermore, the replication of the evolved RNA by Qβ replicase was slightly promoted through the addition of lipids to the reaction mixture. Thus, my primitive cell model demonstrated the evolvability of the genetic material.

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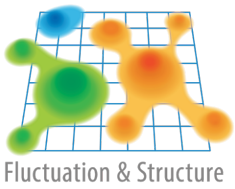
Sponsorship



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