JULY 2013: FIRST ANNUAL REVIEW OF RESEARCH BY THE 2011 WINNERS OF THE "ORIGIN OF LIFE CHALLENGE"

INTRODUCTION

On June 29, 2013, the winners of our Origin of Life Challenge met by videoconference to review the results of the first year's work and to consider proposals for extension of the research.

The three winning teams were:

1. John Sutherland of the Medical Research Council of Cambridge, England, and Matthew Powner of the University College, London, England (the "UK Team").

2. Peter Unrau of Simon Fraser University of Vancouver, British Columbia, Canada; Niles Lehman of Portland State University, Portland, Oregon, USA; and Paul Higgs of McMaster University of Hamilton, Ontario, Canada (the "Canadian/US Team").

3. David Deamer of the University of California at Santa Cruz, Santa Cruz, California, and Wenonah Vercoutere and Veronica DeGuzman, both of the NASA Ames Research Center in California (the "US Team").

Also attending the conference were three Origin of Life experts who volunteered to review the work and provide a report to the sponsor, Harry Lonsdale. They were: Irene Chen of the University of California at Santa Barbara, Nick Hud of Georgia Tech University in Atlanta, Georgia, and Ram Krishnamurthy of the Scripps Research Institute in La Jolla, California. In addition, Jack Szostak of Harvard University, who did not attend the conference, volunteered to review the written reports of the three teams and provide a written review to me.

Present at the conference were the eight members of the three winning teams, the three reviewers cited above, and myself, who served as the moderator of the conference.

The proceedings of the 4-hour conference were recorded and can be viewed by clicking on the link https://bendres.webex.com/bendres/ldr.php?AT=pb&SP=MC&rID=35818957&rKey=e90df2b99ef13072

BACKGROUND

Readers familiar with the RNA World may want to jump ahead to the Results section below.

The Origin of Life Challenge addresses the fundamental question: How did life begin on planet Earth? While there are many creation myths, some going back thousands of years, the most well-known one today is the Genesis story in the Judeo-Christian Bible. But the only honest answer is that we simply do not know. Not only do we not know the "how," we also do not know the "where." Some researchers have proposed that life began in hydrothermal vents on the ocean floor. Others lean toward the "warm little pond," first suggested by Charles Darwin in the 19th Century. Still others have proposed that life (or at least the organic compounds of life) arrived on Earth from outer space. But that only begs the question: How and where was life created "out there" and how did it get from there to here, perhaps from Mars or from other nearby solar systems.

While we don't know how or where, we do have reasonable certainty of when. Most experts believe, based on fossil and other evidence, that life began about 3.8 billion years ago, or some 700 million years after the Earth accreted from the disk of dust and gases swirling around the sun, 4.5 billion years ago.

What were the conditions on the earth at that time, and what chemicals and energy sources were available to drive the reactions that led to increasing chemical complexity? It is likely that the early Earth was much hotter than at present--perhaps 80-90ºC--and the atmosphere consisted mainly of nitrogen (N_2) and carbon dioxide (CO_2), with no appreciable amount of oxygen. (O_2 came later, mostly from photosynthesis, for which it is a waste product.) The elements of the periodic table, from hydrogen to uranium, were present, along with abundant water and many simple chemicals such as hydrogen cyanide (HCN), hydrogen sulfide (H_2S), formaldehyde (CH_20), hydrocarbons, and more. In addition, of course, there was an abundance of silicate and carbonate minerals. For energy sources, there were lightning, heat from volcanoes, and abundant ultraviolet radiation from the sun. UV light is well known to drive a variety of photochemical reactions.

That's what we know today with a certain degree of confidence, but even some of that knowledge is subject to challenge. How did life emerge from such a minimal environment? While we don't know the answer yet, we do know that one major factor was TIME. If life began when the Earth was 700 million years old, that's 250 billion days. In that amount of time, even with minimal starting materials, a great variety of improbable chemistry must have occurred. Some combination of those chemistries led to the first living thing, and it only had to happen once.

What is the level of our understanding today? The evolution of scientific knowledge generally occurs in this sequence: We begin with an hypothesis, the testing of which leads to a model, the refinement of which leads to a theory, the validation of which leads to a law, such as Newton's Law of Gravitation. In the Origin of Life, we are still in the hypothesis stage.

Many hypotheses have been put forward in the past century of Origin of Life research, most of them now discarded. (Those hypotheses are described in the books listed in the Bibliography at the end of this essay.) The hypothesis now in general (but not universal) favor is the "RNA World" hypothesis.

Before discussing the RNA World, we first need a definition of life. For something to be alive, it must be able to carry out two functions. It must be able to reproduce, and it must be able to "live off the land," that is, it must be able to extract useful energy from its environment and use that energy to make copies of itself. In modern cells, that process is called "metabolism."

The RNA World hypothesis goes something like this:

> First, monomers of RNA (ribonucleic acid) were synthesized. The monomers of modern RNA are referred to as nucleotides, but there may have been a more primitive version of the monomers.
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> Second, the monomers formed a variety of polymers by a yet-to-be-discovered process.
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> Third, the RNA-like polymers "evolved" into two useful forms. By "evolved" we mean that selected species were either more chemically stable than other species, or they were better able to utilize the starting materials in their environment. One of the two useful forms was an RNA polymer that encoded useful information in its base sequences, what we would now

call genes. The second useful form was RNA that had catalytic activity, such that it catalyzed essential biochemical processes such as those involved in proto-metabolism.

The three steps above remain unresolved in detail, but there is evidence to suggest that they are at least plausible. A chemical synthesis of RNA monomers was first demonstrated in 2009 by the UK Team of Sutherland and Powner. As discussed under Results below, those researchers are now extending their work using a more plausible mechanism.

A central feature of the RNA World hypothesis is that RNA could have served as *both* the replicator species (the species carrying the genetic information on the early Earth, just as DNA does in modern cellular life) and as a catalyst. To serve in either function requires a degree of complexity not found in a single nucleotide molecule, but polymeric RNA is sufficiently complex to carry information and serve as a catalyst. One key to the Origin of Life puzzle, then, is how do RNA molecules polymerize? As reported in the Results section, two of the research teams have been studying ways that polymerization may have occurred on the early Earth. In addition, the Canadian/US Team has also been studying how catalytic activity in RNA could have emerged from selection of specific nucleotide sequences in random polymers.

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Over the past several decades of Origin of Life research, an argument has been on-going as to which came first: replication or metabolism, proteins or RNA? The RNA World hypothesis has gained support because, while there are still many gaps in our knowledge, it offers a route from non-life to life that seems feasible and is testable by experimentation. The current work supported by our Challenge is aimed at demonstrating the feasibility of key steps in that pathway.

RESULTS

NOTE: The research cited below was carried out by senior scientists in the Origin of Life field. Some of these people began their relevant work 10, 20, or 30 or more years ago. And the work reported here built on much of that earlier work, work that was funded by a variety of government agencies, the most important in the US being NASA.

The work of the three teams is all based on the RNA World hypothesis. The approaches all differ in detail but are complementary.

1. *The UK Team*

John Sutherland and Matt Powner are organic chemists. They are most noted for their landmark publication in *Nature* magazine in 2009, work that was reported in *The New York Times* in May of that year.

The article in *Nature* merits a digression. In that work the authors demonstrated a way to synthesize nucleotides from simple starting materials. Nucleotides are the monomers of which both DNA and RNA are made. Nucleotides consist of three types of chemicals: a type of organic base, a type of sugar, and a phosphate. However, when the base, sugar, and phosphate of which a typical nucleotide is made are combined in aqueous solution, they do not react. Even adding catalysts does not bring about a reaction. But Sutherland and Powner succeeded in carrying out the reaction by combining a sub-unit of the base with a sub-unit of the sugar, then combining the remaining sub-units and finally adding phosphate. It was the first-ever synthesis of a nucleotide "from scratch." (It was that article in the *NYT* that drew my attention and ultimately led me to issue my Origin of Life Challenge in 2011.)

In their work during the past year, Sutherland and Powner took a totally new tack toward synthesizing nucleotides. Traditionally, organic syntheses are carried out by combining two reactants in a vessel--say A and B--and adding some energy to the system (e.g. by heating) to produce product C. In their new approach, called "systems chemistry," the researchers combined multiple reactants at once to produce a variety of reaction products. The chemistry is too complex to describe here. However, by combining very simple chemicals known to be present on the pre-biotic Earth (hydrogen cyanide, hydrogen sulfide, water, and copper compounds) and using ultraviolet light as an energy source, they produced a variety of interesting products, including amino acids, sugars, and lipids, which could have been building blocks for important pre-biotic biochemicals including proteins, nucleotides, and cell membranes. The products even suggest a possible route to proto-metabolism.

Systems chemistry sometimes carries the stigma that, with so many chemicals in the pot and so many reactions going on simultaneously, it's hard to know the specific chemical pathways. However, the investigators have laid out a plausible pathway for each product.

While the results are exciting (and a potential breakthrough) much more work will be required before we will know how these products could have been combined in some orderly way to lead to something approaching the First Life. The results will be published in the scientific literature.

2. *The Canadian-US Team*

This team consists of three investigators at three institutions, searching for the mechanism by which nucleotides could have a) polymerized, and b) once polymerized, randomized their nucleotides. Randomization is essential if these RNA polymers were to evolve to produce catalytic activity (catalytic RNAs are called ribozymes), as well as genetic information. As noted in the Background section above, all of their work is based on the assumption that RNA, in polymeric form, served as both the pre-biotic information carrier and as the catalysts necessary to assist in biochemical reactions.

The Unrau lab has concentrated on how clays, along with certain metal ions which would have been abundant on the early Earth, might assist in the polymerization of RNA, from the types of nucleotides produced from the work of the UK Team. Their results have been positive. By subjecting nucleotide monomers to different pHs and temperature cycling, they have produced stable nucleotide dimers and trimers.

The Lehman lab has been investigating how polymers of RNA can self-disassemble and self-reassemble under pre-biotic conditions. Such disassembling and reassembling into new configurations would be essential in providing variability in the reformed polynucleotide, and variability is essential if these molecules are to evolve into ribozymes. Their results have also been positive.

Paul Higgs is a physicist by training, with a specialty in computational science. His work begins by assuming that replicating RNA molecules were present pre-biotically, and he is studying the conditions that would allow favored RNAs to spread and evolve in competition with unfavored RNAs, ultimately leading to the transition from non-life to life. His work will help guide the design of future experiments by Unrau and Lehman.

All three groups have made reasonable progress in their first year, although the synergy between the groups is still evolving. The path from RNA monomers to RNA polymers and then to useful ribozymes and a piece of a living system remains a challenge.

3. *The US Team*

This team consists of Dave Deamer and two of his former Ph.D. students, Wenonah Vercoutere and Veronia DeGuzman. They are all chemists.

Deamer has been pursuing Origin of Life research for more than 30 years. He has recently written a book on the subject, “First Life” (2011). In 2011, he was elected by his peers to become the president of ISSOL, the International Society for the Study of the Origin of Life.

This team has focused on an entirely different approach to the polymerization of nucleotides and other biomolecules. In their polymeric form nucleotides are held together by chemical bonds that are subject to hydrolysis in the presence of water. The problem in synthesizing polynucleotides then involves either creating those linkages in the absence of water or finding a way to maintain the linkages in the presence of water.

Based on some earlier work, this team investigated the possibility of forming polynucleotides under pre-biotic conditions by exposing the mononucleotides to lipids (organic molecules with a polar and a non-polar end that readily form bilayers) and to hydration-dehydration cycles. The expectation was that the lipids would form bilayers that entrap and organize the mononucleotides within the multilamellar matrix, promoting their polymerization. When the matrix and nucleotides undergo multiple cycles of wetting and drying, the monomers could form increasingly long polymers.

They constructed an apparatus to test this hypothesis. The mononucleotide-lipid mixture was subjected to repeat hydration-dehydration cycles and temperature cycles similar to what is believed to have been present on the early Earth. On analyzing the system after several such cycles, they found clear evidence that polynucleotides had been synthesized. Furthermore, there was good evidence that double-stranded RNA was among the products. If so, this represents a novel result with significant implications for the RNA World hypothesis. The RNA strands of ribozymes must fold into double stranded structures in order to exhibit catalytic activity. Furthermore, the synthesis of double stranded molecules is an essential process in replication.

Toward the end of the year’s work, a group of European researchers in collaboration with Dave Deamer--Marie-Christine Maurel and Laura DaSilva at Universite Pierre et Marie Curie in Paris and Felix Olasagasti at the University of the Basque Country in Spain--carried out similar experiments with similar results.

The results are preliminary and require replication by other laboratories. If confirmed, they provide a possible route to the synthesis of RNA-like polymers, one piece of the Origin of Life puzzle.

CONCLUSIONS

Based on the written reports from the three teams and the June 29, 2013, videoconference, the four expert reviewers all recommended that all of the work be continued for another year. I agree. Thus, the UK Team will be granted $150K, the Canadian-US team will be granted $90K, and the US Team--now the US-European team--will be granted $80K, a small increase from the past year to help support the work of the Europeans.

We still have a very long way to go. Fortunately, many other agencies are supporting Origin of Life research. A major new source of funding was recently announced by the Simons Foundation (www.simonsfoundation.com). Clearly, many more years of research will be required before we have an acceptable model.

It has been obvious for many years that the successful transition from non-life to life on Earth, more than 3 billion years ago, was a “long shot,” a rare event that probably occurred, at least here on Earth, only once. What works in our favor, however, is that, on the order of a billion years passed from the time of the formation of the Earth 4.5 billion years ago until the Origin of Life. Many improbable events can occur in a billion years.

We hope to continue supporting promising Origin of Life research for several more years.

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From time to time, we plan to report on this website the scientific publications resulting from the research we are supporting.

Harry Lonsdale

(Lonsdale is a retired chemist and the co-founder of Bend Research, Inc. of Bend, Oregon [www.bendresearch.com].)

BIBLIOGRAPHY

Dozens of books have been written on the Origin of Life. Here are some that we believe deserve citation.

James Darnell, “RNA. Life’s Indispensable Molecule,” 2011

Paul Davies, “The Origin of Life,” 1998

William Day, “How Life Began,” 2000

David W. Deamer and Jack W. Szostak (Eds.) “The Origins of Life,” 2010

David Deamer, “First Life,” 2011

Christian DeDuve, “Blueprint for a Cell,” 1991

Freeman Dyson, “Origins of Life,” 1999

Iris Fry, “The Emergence of Life on Earth,” 1999

Robert Hazen, “Genesis,” 2005

Stuart Kauffman, “At Home in the Universe,” 1995

Stuart A. Kauffman, “Reinventing the Sacred,” 2008

Naom Lahav, “Biogenesis,” 1999

Pier Luigi Luisi, “The Emergence of Life,” 2006

Harold Morowitz, “Beginnings of Cellular Life,” 1992

Addy Pross, “What is Life?” 2012

Horst Rauchfuss, “Chemical Evolution and the Origin of Life,” 2008

J. William Schopf, “Life’s Origin,” 2002

Robert Shapiro, “Origins,” 1986