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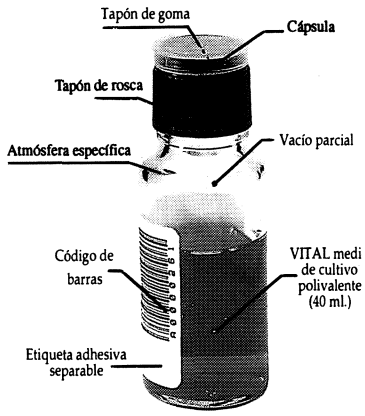

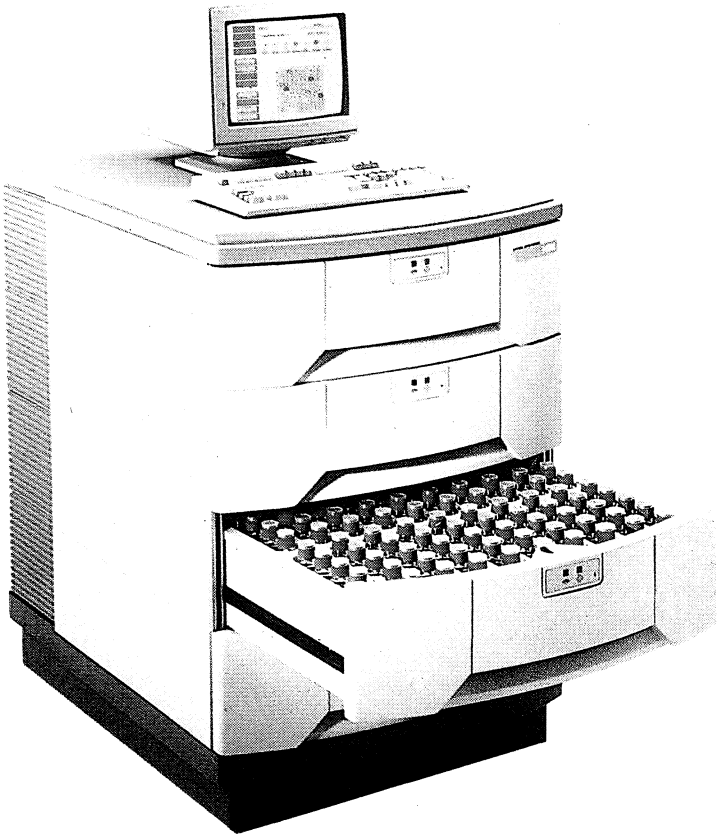

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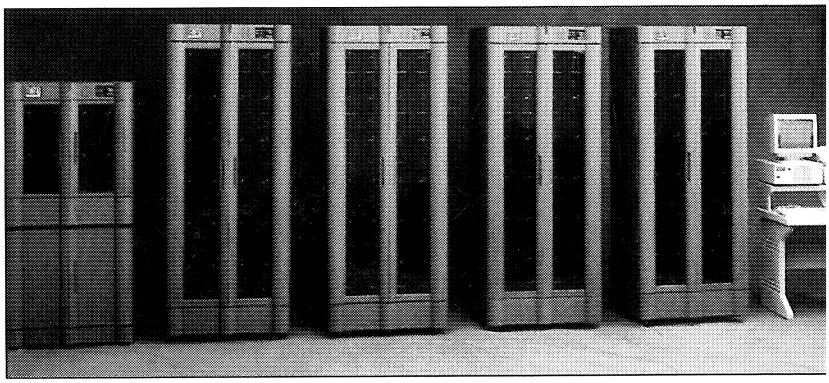

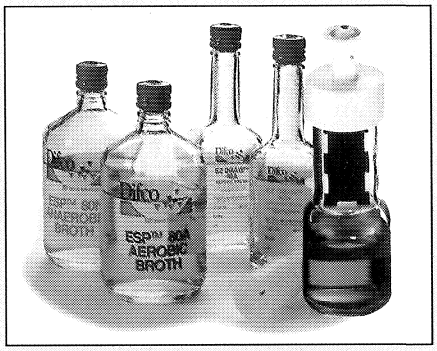

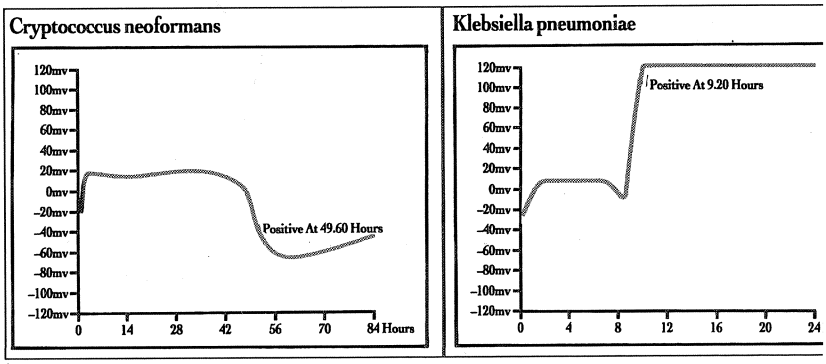

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La revista *Microbiología SEM* y la Sociedad Española de Microbiología agradecen la ayuda recibida de distintas personas y centros de la **Universidad de Barcelona**.

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***Instructions to Authors*:**
Information about the Journal, including instructions on the preparation and submission of manuscripts, is published on pp. 285–286 of this issue, and may also be obtained from the Editorial Office.

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Acknowledgments

We are especially grateful to Mr. Daniel Pagès, Catalan wine producer, and to the Catalan Foundation for Agriculture—of which Mr. Daniel Pagès is the President—for their generosity, which has allowed the publication of these materials. Without their sponsorship, the release of this special issue of *Microbiología SEM*—devoted to ISSOL'93 meeting—would not have been possible.

We express our thanks to the Spanish Society for Microbiology for having allowed us to publish in its journal, *Microbiología SEM*, the articles which have made up this special issue. We acknowledge Ricardo Guerrero, editor-in-chief and Jordi Mas-Castellà secretary of *Microbiología SEM*, for his continuous assistance and effort in the preparation of the papers presented here. We thank the authors who have worked hard and enthusiastically since we asked them the articles, as well as the anonymous reviewers for their help.

The issue could not have been completed without the dedicated help of the publication board of *Microbiología SEM*: Carmen Chica, Mercè Piqueras, Ana F. de Castillo, Eulàlia Massana. We gratefully acknowledge Stephanie Hiebert, Susana Benedito, Meritxell Riquelme, Ana B. Paules, and the students at the University of Barcelona, who transcribed several recorded lectures from ISSOL'93, as well as Michael Dolan, who edited several of the articles.

To all of them, our deep thanks.

Joan Oró and **Antonio Lazcano**
Invited editors

MICROBIOLOGÍA SEM **11** (1995), 143–144

Presentation

The 1st Meeting of the International Society for the Study of the Origin of Life (ISSOL) corresponding to the 4th International Conference on the Origin of Life were held in Barcelona (Spain) in June, 1973. Since then, ISSOL members have met in Kyoto (1977), Jerusalem (1980), Mainz (1983), Berkeley (1986) and Prague (1989). In July 4 through 9, 1993, the meeting was held again in Barcelona. Several circumstances coincided that made the 1993 meeting in Barcelona (ISSOL'93) particularly successful. First of all, we celebrated the 20th anniversary of the first ISSOL meeting. Secondly, ISSOL commemorated the centenary of Harold C. Urey's birth (1883–1981) by establishing the Harold C. Urey Medal for scientific excellence in the studies of the origin of life. In the future, this medal will be awarded alternately with the A. I. Oparin Medal at the triennial meetings. Leslie E. Orgel was the first recipient of the Urey Medal.

ISSOL'93 transcended the province of science and scientists in the multitude of media coverage which occurred, providing the opportunity for non-scientists to learn about the gathering of researchers from around the world to discuss the origin of life, a question of intrinsic interest to all humankind.

From a scientific point of view, ISSOL'93 was a very fruitful meeting. The attendants were fortunate enough to have excellent lecturers in all the symposia and sessions. Some of the lecturers at the meeting are presenting their latest work on the origin of life in this special issue of *Microbiología SEM*. We thank both the Spanish Society for Microbiology and Prof. Ricardo Guerrero, Editor-in-Chief of *Microbiología SEM*, for providing us with the forum of the scientific community that this journal represents. In this issue, additional papers—contributed by colleagues who were unable to present them at the ISSOL meeting—with high quality and related to the topic of the origin of life, have been incorporated. We consider the contributions to this issue to make a complete and updated view of the state of the art in the study of the prebiotic chemistry and the later evolution of living forms.

The papers presented here follow the chronological order of the events which they deal with. Both J. Oró and S. L. Miller are contributing with review papers on cosmochemical evolution and the prebiotic synthesis of organic compounds respectively. L. Margulis and R. Guerrero are presenting a paper which considers life as a planetary phenomenon, which might take place not only on Earth, but also on other planets. P. E. Nielsen is explaining the use of novel DNA analogs as models for the primordial genetic material. G. E. Smith and G. E. Fox are approaching the study of primitive replication. P. Forterre

is presenting his work on the the use of the enzyme reverse gyrase to argue that hyperthermophiles can not be the first forms of life. T. G. Tornabene is contributing to this issue with a paper on the biogenic origin of hydrocarbons; this paper is based on a lecture given in January 1994 at the University of Houston, Houston, TX, as part of the mini-symposium held in honor of Dr. J. Oró. T. G. Waddell and G. K. Bruce summarizing their ideas on the origin and evolution of the citric acid cycle. A. Lazcano attempts to demonstrate that the last common ancestor of all extant living beings must have been a bacterium-like cell. R. Amils and E. Sánchez are approaching the study of ribosomal evolution by using functional inhibitors such as antibiotics. J. Peretó has written an article to commemorate the centennial of the birth of A. I. Oparin (1894–1980). M.-C. Maurel is presenting a paper which links the topic of this issue with the ideas of Pasteur, whose death's centenary is being commemorated this year. R. A. Goldsby has written the obituary of Cyril Ponnamperuma (1923–1994), former ISSOL President.

The usual session on books reviews is devoted to the most recent books dealing with the studies of the origin and early evolution of life, some of them having been published even after the ISSOL'93. As a complement to the reviews, there is a list of books available on the origin of life published either in English, Spanish or Catalan.

The high standards of the ISSOL'93 were the result of the efforts of many people and institutions. We wish to thank the support and collaboration received from various institutions such as the Autonomous Government of Catalonia (Generalitat de Catalunya), the City Council of Barcelona, the Catalan Research Foundation, the Catalan Society for Biology, the University of Barcelona. We are very grateful to Mr. Manuel Pagès, former President of the Codorniu sparkling wine ("cava") cellars, for the fabulous tour and reception at the cellars for the mid-congress excursion. We must especially thank Mr. Daniel Pagès, a major Catalan wine producer and philanthropist, for his generous financial support of several activities of ISSOL'93. Without his help, ant the support received from the Catalan Foundation for Agriculture, presided by Mr. Daniel Pagès himself, the publication of this special issue of *Microbiología SEM* would not have been possible.

Professor Néstor Hladun, the Dean of the School of Biology of the University of Barcelona, and his technical staff, under the expert leadership of Mr. Agustí Bonet, provided invaluable services for ISSOL'93. Of course we cannot forget the members of the Local Organizing Committee, as well as the enthusiastic efforts of the 25 students, the "issolitos" ("ISSOL's little folks"), as they called themselves. The participants were happy to have their cheerful and efficient help, and the "issolitos" received letters of thanks from around the world.

Last, but not least, we are particularly indebted to especially thank the Publication board and staff of *Microbiología SEM*, which, on this special occasion, counted on the invaluable collaboration of Michael Dolan for the editing of the articles. Finally, we wish the best to André Brack, the organizer of ISSOL'96 in Orleans (France).

Joan Oró and **Antonio Lazcano**
Invited editors

MICROBIOLOGÍA SEM **11** (1995), 145–160

Cosmochemical evolution and the origin of life

Joan Oró

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Summary

Since hominids became conscious of their own existence and developed the ability to think, they were intrigued by the immediate and sidereal environment around them. As soon as they realized that they would eventually cease to exist as individuals, they became profoundly concerned about fundamental questions regarding their own existence. One of the major questions was where do we come from, and, by extension, what is the ultimate origin of all life on Earth. Leaving aside old, often religious, historical accounts which embody this primal question, it was not until the last two centuries that attempts to treat this matter in a scientific way have been made. The present very limited review summarizes the current status of the origin of life problem as an experimental scientific subject since it was treated in this way for the first time this century by Oparin.

Key words: origin of life, cosmochemistry, interstellar molecules, prebiotic synthesis, evolution

Resumen

Desde que los homínidos fueron conscientes de su propia existencia y desarrollaron la habilidad de pensar, quedaron intrigados por el medio inmediato y sideral que les rodeaba. Tan pronto como reconocieron que con el tiempo dejarían de existir como individuos, se preocuparon profundamente de las cuestiones fundamentales referentes a su propia existencia. Una de las más importantes es de dónde venimos y, por extensión, cuál es el origen último de toda la vida en la Tierra. Dejando explicaciones históricas, a menudo con connotaciones religiosas, que incluyen respuestas a esta pregunta, únicamente en los dos últimos siglos se ha intentado tratar este tema de un modo científico. La presente revisión

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resume brevemente el estado actual del problema del origen de la vida como una materia científica y experimental desde que fue tratada de este modo, por primera vez en este siglo, por Oparin.

Introduction

Among the most basic problems confronting science are those regarding the origin of the universe, the origin of life and the origin of man. The problem of the origin of life on Earth has concerned man since the beginning of history, but until relatively recently this has been an issue that has been of more interest to philosophy and religion than to science. It is not surprising, therefore, that ideas on this subject have been accepted, in the past, without either critical analysis or logically sound corroboration. In the last century, Charles Darwin's observations on the evolution of species (25), established a solid base for a possible approach to this question through the application of the scientific method.

The fundamental problem of the origin of life on Earth was not treated following the scientific method until Aleksandr Ivanovich Oparin proposed his revolutionary theory of chemical evolution (63, 64, 65). The studies made during the last four decades, including micropaleontological discoveries of ancient microfossils, observations about the cosmic environment of the Solar System and our galaxy, and, particularly, laboratory experiments on the prebiological synthesis of biochemical compounds, gave support to the hypothesis of chemical evolution as a scientific explanation for the origin of life on Earth.

Scientific approaches and Oparin's views

From a scientific point of view, the origin of life question may be approached from two different angles. On one hand, this may be done by historical and biological studies which provide essentially an analytical approach. This involves the micropaleontological search for the oldest terrestrial rocks in order to find the most ancient organisms in the form of microfossils, thereby leading to the elucidation of the circumstances of the appearance of life on Earth. This line of inquiry is also complemented by the study of the evolution of biomacromolecules in extant cells in order to ascertain the nature of the earliest living systems.

The other angle is cosmic and more chemical. It requires observational studies on the composition of the interstellar medium and the Solar System, followed by a synthetic experimental approach. More specifically, it involves, first, cosmochemical and geochemical studies to determine the conditions of the primitive Earth which led to the appearance of the first living forms, and second, laboratory studies intended to reproduce the pertinent fundamental chemical processes of biomolecular synthesis and organization.

According to Oparin's views, the sequence of events in this chemical evolutionary process started with the abiotic formation of simple organic compounds such as the biochemical monomers which today constitute the proteins and nucleic acids of living systems. These monomers reacted to form polymers which in turn interacted to generate molecular structures of increasing complexity until an entity was formed that could be called "living". The fundamental and distinctive aspect of this hypothesis is that

it calls for a step by step process of chemical evolution, where the chemical complexity of the molecules or structural units involved, increases gradually and progressively with time.

Experimental support

Unfortunately, Oparin's book, which was published in Russian in 1924 (63), was not translated into English until much later (64, 65) and therefore, received little attention until the 1950's, when Harold C. Urey (98) presented similar views about the reducing state of the terrestrial atmosphere in relation to the problem of the origin of life. This may explain why the first synthesis of organic compounds under possible primitive Earth conditions, was not carried out until 1953, when Stanley L. Miller was able to synthesize amino acids from a mixture of methane, ammonia and water (53, 54, 55, 56).

In addition to the α-amino acids, which are the monomeric units of proteins, it was also reasonable to attempt to provide further experimental support for Oparin's theory by synthesizing abiotically some of the purine and pyrimidine bases which are part of the nucleotides. This was not accomplished until 1960 when adenine, and later other purines, were synthesized under possible primitive Earth conditions from the hydrogen cyanide, ammonia and water mixtures in my laboratory (69, 70, 71, 72).

Once the building blocks of the two major groups of biomacromolecules had been synthesized prebiotically many other experiments were undertaken by scientists interested in these studies. There are many questions still to be solved, but at the present time, this analytical and experimental research approach seems to provide the most rational and coherent scientific framework to explain the natural origin of life on Earth. This paper is a very brief non-comprehensive summary of what has been accomplished during the last four decades concerning the observational and experimental aspects of the study of the origin of life on Earth. More detailed reviews about the prebiotic synthesis of biochemical compounds in relation to the origin and evolution of life on Earth may be found in other publications (32, 57, 58, 81).

Biogenic elements and interstellar organic molecules

Before considering the conditions of the primitive Earth, it is necessary to go back in time and discuss briefly the cosmic formation of the biogenic elements. These are H, C, N, O, S, P and other trace elements. About 98% of the matter of the universe is made of hydrogen and helium. Essentially all the elements are synthesized in stars (2). Four nuclides of hydrogen are condensed into an α particle by means of a thermonuclear reaction which occurs in the nuclei of stars like our Sun at about 15 million degrees. This is an exergonic process which in accordance with Einstein's equation generates the energy that has allowed the appearance and continuation of life on our planet for about four thousand million years.

From a biological point of view, perhaps the most significant thermonuclear reaction is the next one, whereby three α particles are condensed into a nuclide of carbon. This occurs in carbon-rich stars at about 100 million degrees. Without carbon, life, as we know it, would be impossible. Other thermonuclear reactions start with the carbon nuclide and generate all the other biogenic elements (2), from which many interstellar molecules are formed (40, 72, 75). These interstellar molecules are considered to be the precursors of the Solar System and in a certain sense life itself.

The biogenic elements are combined in the exterior of the atmospheres of cool carbon stars and give rise to the formation of simple and complex organic molecules. The first organic molecule detected in the interstellar medium of our galaxy was formaldehyde. Interestingly, the first five molecules discovered in interstellar space were ammonia, water, formaldehyde, hydrogen cyanide, and cyanoacetylene, from which one can synthesize in the laboratory most of the amino acids and nucleic acid bases necessary for life. Since then, some 100 new molecules have been detected. About 75% of them are organic, and the rest are inorganic, so that the universe is essentially organic and prepared for life to appear when the conditions are right, as must have happened on the primitive Earth (74, 84).

Formation of the Solar System

The Solar System was generated about five thousand million years ago by the gravitational collapse of the Solar Nebula when a critical mass was reached. This collapse is supposed to have been triggered by a shock-wave from a neighboring supernova explosion. Thus, the protosun, the protoplanets, the comets, the parent bodies of meteorites, and other planetesimal bodies were formed as the result of this condensation of interstellar matter. Subsequently, as evidenced by the lunar cratering record, during several hundred million years, the Solar System appears to have been in a state of great upheaval where the norm was the continuous collisions of planetesimals with other major bodies such as the planets.

The composition of the Solar Nebula reflected that of the interstellar clouds out of which it was made. This means that it consisted primarily of hydrogen, helium, and organic compounds. This has been confirmed by astronomical studies of the giant planets, Jupiter and Saturn, and their satellites. A typical example is given by the composition of Titan, a major satellite of Saturn. The atmosphere of Titan was discovered by Josep Comas Solà from the Fabra Observatory in Barcelona in 1907. More recently, and by means of the NASA Voyager spacecraft, the atmosphere of Titan (86, 90) has been demonstrated to be made mainly of nitrogen and methane with small amounts of hydrogen cyanide, cyanoacetylene, and other organic compounds, some of which are also present in comets. The D/H ratio of Titan is identical to that of Halley's comet and its dense atmosphere was presumably formed by the capture of comets (102). The collision of comet Shoemaker-Levy 9, with the planet Jupiter in July 1994, is considered to be a major cosmic event this century (37).

Comets and carbonaceous chondrites

Comets are interesting small bodies of the Solar System from the point of view of the origin of life. They are considered to be the most pristine and primitive bodies of the Solar System and presumably contain molecules, from ancient interstellar clouds, still frozen at the low temperature of interstellar space. The presence of hydrogen cyanide, formaldehyde, and other more complex organic compounds such as adenine, which can be formed from hydrogen cyanide, has been demonstrated in cometary dust by the PUMA and PIA mass-spectrometers on board of the Vega and Giotto spacecrafts, respectively, which analyzed comet Halley in 1986 (42).

The carbonaceous chondrites are derived from dark asteroids and contain complex organic molecules. In the Murchison meteorite, which fell in Australia in 1969, some seventy five amino acids

were found. Eight of the amino acids such as glycine, alanine, and glutamic acid for example, are also found in proteins. A few others are also of metabolic interest, such as *gamma*-aminobutyric acid (GABA), but many of them have not been found in biological systems. Furthermore, all of them are racemic (equal mixtures of D- and L-isomers) which together with other properties, i.e. the D/H ratio, suggests that these amino acids were chemically synthesized from extraterrestrial precursors, which precede the formation of the Solar System (21, 23, 82).

Formation of the Earth-Moon system and the origin of volatiles

Because of the turbulent state of flux during the formation of the Solar System, the proto-Earth was not exempt from collisions. A recent model proposed by Cameron and Benz has led to a new theory (16). According to Cameron, a body with mass similar to that of Mars collided with the proto-Earth, injecting most of the iron into the nucleus of the Earth, and causing the fusion and ejection into orbit of part of the mantle which eventually coalesced to form the Earth's only natural satellite. In this way, most of the similarities and slight differences between the composition of the Earth's mantle and the Moon are explained, such as, the angular momentum of the Earth-Moon system and the iron-poor nature of our natural satellite. The impact of the collision was such that the light elements and volatile compounds such as water, were lost into interplanetary space.

However, this presents the question which the author asked Dr. Cameron: What was the source from which the Earth did get all the water and light elements in order for life to appear on our planet? In a very simple way, Cameron answered "from comets and other small bodies of the Solar System which collided with the Earth, in the latter phases of terrestrial accretion". This is in line with a theory proposed by the author in 1961 (70).

The above two theories appear to be accepted by the majority of scientists working in this field today. Namely, that the Earth-Moon system resulted from a massive collision, losing most of the water and volatile elements into space, and that comets and other small bodies of the Solar System subsequently contributed by late accretion processes the water and most of the biogenic elements through collisions which took place during the first 600 million years of Earth's history.

In the initial paper (70), it was calculated that the amount of cometary matter captured by the Earth was up to 10^{18} g, which is approximately the mass of all the biosphere. Later, we improved our calculations and we arrived at the amount of 10^{23} g (78). More recent calculations by Chyba, Sagan and collaborators have resulted in a range of similar numbers (24). Other calculations by Whipple and Delsemme, two cometary astronomers, and other investigators have produced values of the order of 10^{25} g, which would indicate that even all the water in our oceans is from cometary origin (27, 28, 39, 83). Indeed, the D/H ratios of Halley's comet and those of sea water are essentially identical (8).

Prebiotic synthesis of amino acids

As pointed out, Oparin was the first to propose that a long period of chemical abiotic synthesis of organic compounds is a prerequisite for the appearance of the first life forms on the Earth.

The first experiment which successfully tested Oparin's hypothesis that biochemical compounds could be synthesized chemically under prebiotic conditions was performed by Miller (53, 54, 55, 56), who worked with Urey at the University of Chicago. From a mixture of methane, water and ammonia and by means of electric discharges, he was able to obtain a number of the amino acids which are the building blocks of proteins.

Before this discovery, one of the first experiments to put Oparin's theory to the test had been performed by Garrison et al. (34). They used carbon dioxide and high-energy radiation. This experiment did not yield very encouraging results. It was then that Urey suggested, at the University of Chicago, that it would make more sense to use a more reduced carbon compound, such as methane, just as Oparin and Urey himself had proposed earlier (98). It was Miller, who through the use of electrical discharges and a more reduced carbon compound (methane) in ammonia and water, obtained α–amino acids in an experiment which is now considered classic.

As is now well-known, Miller's experiments identified several of the amino acids that are found in proteins, such as glycine, alanine, and glutamic acid. Additionally, hydroxy acids, other biochemical compounds, and non-proteinic amino acids were also formed. It is quite noteworthy that the mixture of synthesized amino acids was found to be similar to that of the Murchison meteorite in both composition and racemization, i.e. both mixtures had equal ratios of each of the optical isomers of both the amino acids and the hydroxy acids formed. Thus, it was concluded that the mechanism of synthesis, the Strecker-cyanohydrin condensation, was probably the same on the primitive Earth and in the parent bodies of the carbonaceous meteorites (82, 87).

Synthesis of adenine and other purines

The second major question related to the formation of biomonomers on the primitive Earth involved determining how purines and pyrimidines, the bases of nucleic acids, could be synthesized abiotically. Convincing evidence that hydrocyanic acid produced adenine in the presence of ammonia and water was obtained in 1960 (69). Not only was adenine obtained, but other purines, amino acids and other biochemical compounds were also produced by the reaction (69, 70, 71, 72, 73). In previous biochemical work at Baylor University College of Medicine, I was able to isolate and characterize purines and some of their intermediates such as aminoimidazolecarboxamide (AICA). Thus, when I conducted the synthesis experiments at the University of Houston, I had the great satisfaction of seeing that AICA, as well as other intermediary compounds, had also formed abiotically from hydrocyanic acid. That is to say, it was then possible to conceive a prebiotic synthesis mechanism (71) which bore some resemblance, as far as the key intermediates were concerned, to the biological one.

We soon made a detailed study of the synthesis of adenine (79, 80). Using nine different methods, we were then able to prove that the product synthesized was definitely adenine, thus obtaining the necessary physical and chemical identification. What had at first struck me as a surprise, I later came to see as inevitable, since the empirical formula for adenine corresponds to that of pentameric hydrocyanic acid $(HCN)_5$. It remains a paradox that hydrocyanic acid, one of the most toxic molecules to living systems, had apparently been essential to the prebiotic production of one of the major molecules for life, adenine. Corroboration from the laboratories which had been most skeptical, mainly in Cambridge,

England (52) and Japan (99), was not slow to arrive. A Japanese company modified the original procedure (69), removing the water and obtaining a 15% yield of adenine, a method that was later patented for the commercial production of adenine.

From a conceptual point of view, this discovery had favorable consequences for the generalization of the chemical evolution process. Thus, the synthesis of adenine from hydrogen cyanide, the presence of this compound in comets, and the atmospheric explosion which occurred in Tunguska, Siberia, in 1908, caused most likely by a comet (33, 43, 44, 76), were the three factors that led to propose the hypothesis of the role of comets in the formation of biochemical molecules on primitive Earth (70).

After these first two abiotic syntheses of biochemical monomers, amino acids and purines, many scientists became interested in an experimental approach to the study of the origins of life on Earth, in line with Oparin's chemical evolutionary theory. This rather wide field could be summarized by saying that many biochemical monomers, which form part of living beings and which were necessary for the emergence of life on Earth, have been synthesized abiotically in various laboratories around the world (32, 57, 58, 81). Other interesting abiological syntheses of nucleic acid constituents, including deoxyribose, were accomplished (73, 89). ADP and ATP were also obtained by means of ultraviolet light and some condensing agents (89).

Synthesis of biopolymers

Aside from the relatively easy formation of biomonomers during the first phase of chemical evolution, it is essential in order to understand the whole process of prebiotic evolution, to know what conditions prevailed during the second phase of organic synthesis which led to the formation of the first biological polymers. The conditions needed for this second phase must have been more moderate, since relatively fragile molecules were involved.

Under these moderate conditions, that is to say, temperatures between 0 and 100°C, it has been possible to synthesize oligopeptides and oligonucleotides in the presence of condensing or activating agents in several laboratories. For instance, one can achieve the polymerization of deoxyribonucleotides into oligonucleotides and amino acids into polypeptides, with the help of the condensing agent cyanamide (38, 95, 96) which had been proposed earlier (72) and was found later in the interstellar medium (40). Most of these procedures have been conducted through the evaporation of aqueous solutions. On the primitive Earth, these evaporation processes probably occurred as a result of the daily cycles of night and day. It is therefore conceivable that, due to the continued repetition of the processes of evaporation (day) and cooling (night), relatively high degrees of polymerization could be reached. This is one of the ways to explain the paradox of the polymerization of nucleotides and amino acids, as opposed to the hydrolysis of the same polymers.

Regarding the formation of oligopeptides, an interesting laboratory finding is the synthesis of the dipeptide histidyl-histidine (94), which acts as a moderate catalyst in several prebiotic reactions of synthesis and hydrolysis. It is possible that, by following this line of research, one would be able to synthesize proto-enzymes, or rudimentary enzymes. In our laboratory, we were also able to obtain, through the use of cyanamide, various coenzymes such as adenosine triphosphate (ATP), adenosine diphosphate glucose (ADPG), uridine diphosphate glucose (UDPG), cytidine diphosphate choline (CDP-choline), cytidine diphosphate ethanolamine (CDP-ethanolamine) and other coenzymes (81).

Experiments on the replication of nucleic acids have been conducted in Orgel's laboratory. For instance, oligo G was synthesized using a poly C mould and activated substrates, such as 2-Me-Imp-G, which is the 2-methyl imidazolide of guanosine monophosphate (15). This demonstrated the principle of information transfer at a molecular level by purely chemical means. That is, a negative molecular copy (oligo G) can be synthesized from an original positive molecule (poly C), a reaction that constitutes only one half of the replicative process. So far, the autocatalytic process for complete molecular reproduction of polynucleotides has not been solved, with the exception of certain reactions of hexadeoxyribonucleotides (and tetraribonucleotides). In spite of the chemical difficulties of the nucleotide replication process, substantial progress has been made in this field (66, 67, 92).

The RNA world

The idea that RNA preceded DNA as a genetic molecule has been expressed by many writers and has been summarized by Lazcano et al. (49). At the same time, discoveries by Sidney Altman (1) and Thomas R. Cech (20), for which they were awarded the Nobel Prize, show that certain ribonucleic acids are capable of acting as catalysts. Because of this, they have been called RNA enzymes or ribozymes. This discovery marked the first time in the history of biochemistry that catalysts have been found that are not exclusively proteins. This new catalytic property revealed by certain ribonucleic acids opens the door to prebiotic experiments in a field which Walter Gilbert has christened with the name of the RNA world (35), meaning that life could have existed before the appearance of DNA and proteins. So far, however, the catalytic properties of RNA are relatively limited, and although various experiments on the evolution of the catalytic RNA, or ribozymes have been carried out in vitro, enzymes are still needed to conduct most of these experiments in an efficient way. The work in this field, as shown by recent models and experiments by Cech (18, 19, 88), Doudna and Szostak (29), and Joyce (41), appears to be very promising. Szostak has suggested that certain catalytic activities may also be shown by DNA oligonucleotides.

The appearance of membranes

The origin of life did not depend on the chance appearance of a single molecule. It depended on the gradual evolution of systems formed by groups of biochemical molecules with different properties (reproductive, catalytic, energetic, etc.), but which were mutually interdependent. It is therefore obvious that a mechanism to hold them together within protocellular membranous structures is absolutely necessary. In other words, and in accordance with Oparin (63, 64, 65), Haldane (36), and Bernal (12), the appearance of polymolecular systems associated with membranes must have constituted a decisive step towards the origin and cellular evolution of life. As stated by Bernal, "the formation of membranes must be taken into account in all the comprehensive pictures of the origin of life" (12).

Aside from the question of whether life can exist only in the presence of proteins, or in their total absence, as in the world of RNA, what is certain is that if evolution is to occur according to Darwin's theory, a cell delimited by its membrane is needed in order to separate the intracellular from the extracellular medium. It does not matter how rudimentary this membrane is.

Cell membranes consist mainly of molecules of amphiphilic lipids. These are primarily hydrophobic molecules that are able to assemble themselves spontaneously into liposomes, consisting of a single spherical lipid bilayer surrounding an aqueous core, thus forming microstructures about the size of a cell (1 to 10 μm). Two of these amphiphilic molecules are phosphatidylcholine and phosphatidylethanolamine. In experiments conducted in our laboratory over several years, we have been able to synthesize, not only the building blocks of amphiphilic lipids, namely fatty acids (50) and glycerol, but also specific amphiphilic lipids including phosphatidic acids (30), phosphatidylcholine and phosphatidylethanolamine (85). In the case of phosphatidylcholine, we were able to easily obtain liposomal vesicles similar to cellular membranes, that is to say, having the same type of bimolecular layers as are found in most cells of prokaryotic and eukaryotic organisms. The prebiotic formation of lipids and the encapsulation of macromolecules by lipid vesicles under simulated prebiotic conditions has been undertaken in Deamer's (26) and other laboratories (7).

Protocellular models

This is one of the most difficult aspects of experimental research into the origin of life. Even if we were able abiotically to synthesize RNA which was capable of replicating itself, and we were able to obtain peptides following the instructions of RNA, and even if these peptides were able to catalyze the synthesis of the initial RNA molecule, thus closing the vital cycle (replication, translation and catalysis), two further processes would still be needed: the structural self-assembly of these molecules within a liposome, or protocellular enclosure, and the provision of the energy necessary to initiate and maintain the autocatalytic processes involved with the functioning and then the reproduction of this protocell.

Although Noller et al. (60) have indicated that one of these catalytic RNA molecules could be converted evolutionarily into ribosomal RNA (rRNA), it is still too early to make very specific conjectures about protocellular models. At any rate, three possible evolutionary protocellular models of increasing complexity could be speculated to be as follows. The first is based on the self-assembly of a catalytic RNA within a liposome. The second model contains, in addition to the above, a tRNA molecule capable of synthesizing a catalytic peptide. The third model includes also DNA and a protoribosome. This latter model could be in principle the expression of Darwin's ancestral cell. Certain aspects about the world of RNA and about the transition from non-living to living have been recently discussed by Lazcano (47, 48), in an attempt to elucidate the characteristics of extant life's earliest common ancestor.

ATP and the triggering of life

Even if we accept that the self-assembly of any of the three previously described models can be achieved, one question still remains unanswered. How does a protocell actually start to function? Obviously, each of the three models must receive the necessary energy to bring about the continued synthesis of all its molecules and finally reach the protocellular duplication process, or autopoiesis. In contemporary cells, this energy is provided by pyrophosphates, or ATP, and the formation of a high-energy bond can be generated by a gradient of protons, produced either directly by the conversion of chemical energy, or by light through a suitable pigment (100). Extensive work on the

bioenergetic mechanisms involving inorganic pyrophosphate, as well as ATP, has been carried out by M. Baltscheffsky and H. Baltscheffsky (9, 10).

Life could be initiated in a protocell by a flow of protons (positive ions), which would have induced the formation of ATP (or pyrophosphate), whose energy would then have been used to trigger and generate synthetic reactions in the protocell. Experiments using gradients of protons through the membrane of a protocell, or bilamelar liposomes, might well demonstrate the validity of this principle.

Outstanding questions to be solved

Not everything is rosy in the field of the origin of life. There are a number of serious problems that have not yet been solved. Primarily, those concerning the chemical reactions necessary for the emergence of life on Earth. One is the efficient formation of ribose in order to make RNA, and deoxyribose for DNA. The two compounds can be synthesized abiotically, but questions have been raised, particularly concerning the synthesis of ribose (93). However, some interesting new approaches may eventually be successful. Recent suggestions involve the formation of racemic-ribose-2,4-diphosphate (59) and other compounds.

A second question is that of the chirality of biomacromolecules. Chemical synthesis inevitably leads to a racemic mixture. The cosmic physical solutions that have been offered do not provide an answer, because temperatures of at least 0 to 100°C did prevail on the primitive Earth so that any preformed chiral monomers would be quickly racemized (6). Besides, the facts are that the amino acids in meteorites are racemic mixtures (45). Some excesses of D-amino acids have been found in a few carbonaceous chondrites, but they were due to contamination (5). The solution to this problem will probably be found in the selective formation of homochiral oligomers, because of their increased stability or increased efficiency in their catalytic activities, or structurally dependent functions. A third question concerns the activation of amino acids and the translation of the coded RNA information into peptides. Small RNA oligomers in the form of hairpin structures have been suggested as models (11), but the pertinent experiments have yet to be carried out successfully.

A fourth question is concerned with the formation of straight chain fatty acids of sufficient length. More work needs to be done to improve the yields of these compounds synthesized by Fischer-Tropsch processes (61) or other abiotic pathways, as they are suggested by the presence of branched alkanes in carbonaceous chondrites (22) or cyano-oligoacetylenes in the interstellar medium (40). Long chain fatty acids are necessary for the formation of the amphiphilic lipids of membranes. The fifth major hurdle concerns the self-assembly of a minimum number of the necessary coding and catalytic molecules within a protocell and the triggering into action and autopoiesis of this protocell (31). It is obvious that none of the above relatively complex reactions will be clarified or experimentally solved without spending a considerable effort in attempting their solution.

Early evolution of life

As mentioned earlier, the study of the origin of life can be approached from an evolutionary biological point of view. The most ancient fossils are those found in sediments and rocks in western

Australia (the Warrawoona Formation). These fossils are 3500 million years old and their morphology is similar to cyanobacteria, as shown by Awramik, Schopf and coworkers (3, 4, 91). However, they cannot represent the oldest organisms on Earth because they are too complex, or too evolved. Of the three contemporary unicellular classes (archaeobacteria, eubacteria, and eukaryotes), the oldest are probably thermophilic archaeobacteria which live at high temperatures in the hot springs at the bottom of oceans. But those organisms are not widely believed to be the ancestral cells of all living beings either (47, 48).

It is to be hoped that by studying the sequences of some of the most primitive enzymes which are most preserved in ancient organisms, we shall be able to establish the root of the philogenetic tree of the three great unicellular classes, as is being pursued by Lazcano (47, 48). Solving this problem will not be easy, as the enzymes of any given unicellular organism existing today have had more than 3500 million years to evolve and many changes may have occurred in the process, even in sequences that today seem very well preserved. However, recent progress in molecular biology raises hopes regarding the application of this method to the study of the origin of life.

Biological exploration of Mars

Exactly seven years later after Apollo 11 spacecraft had landed on the Moon, the first Viking spacecraft landed on July 20, 1976, on the surface of Mars. The dust of Mars was red, in keeping with the popular name by which this planet is known. Molecular analysis for volatizable organic and inorganic compounds were carried out using an instrument (GC–MS) that was, in principle, similar to the one used in our laboratory for the lunar sample analysis. The new miniaturized apparatus was built according to the suggestions of the members of the Viking project molecular analysis team. The major difference between the GC–MS instrument in our laboratory and that on the Viking Mars lander, was that the latter, instead of weighting two tons, weighted only 20 kg. What were our findings? We found no organic matter, not even in parts per million, in either of the two landing sites on Mars, the plains of Chryse and Utopia, where the Viking landers actually landed (14).

On the other hand, one of the three biological experiments pointed to the rapid formation of the radioactive carbon dioxide. Levin and Straat suggested that this was the result of considerable microbial activity (51). This may be due to a relatively simple chemical reaction, in which the iron oxides and hydrogen peroxide in the analyzed Mars samples had oxidized to CO_2, the radioactive formic acid which Levin and Straat had included, among other metabolites, in the nutrient solution. It is interesting to note that formic acid, when in the presence of hydrogen peroxide, is rapidly oxidized into carbon dioxide not only by enzymes such as catalase, which have an iron atom in their active center, but also by inorganic catalysts such as iron oxides (Fe^{2+}, Fe^{3+}), which are very abundant on Mars. Furthermore, additional laboratory studies on the oxidation of organic substances by amounts of UV light, which are comparable to those which fall on the surface of the red planet, explained the total absence of organic matter on Mars (68). The half-life of residence of any meteoritic or cometary organic matter exposed on the surface of Mars is barely a few months, an instant in the geological time scale.

We can thus say that there is no evidence for life at the two Viking landing sites on Mars, although it could be a worthwhile proposition for future space missions to revisit the planet in order to look for fossils which would show if life has ever existed there in the past. It is unlikely, although not totally

impossible, that life may exist in some unique sites of Mars, such as a deep crevice or underground thermal spots where some of the ice of the permafrost may be temporarily converted into liquid water.

Life beyond the Solar System

It is reasonable to think that life can exist in other planetary systems. An example of this may well be the orbital system around the star Beta-Pictoris, which is some 54 light years away from Earth. This star is intriguing because it is surrounded by a great ring of comet-like matter which emits intense infrared radiation. It was first detected by the infrared telescope of the IRAS astronomical satellite, and photographed from the Las Campanas Observatory in Chile by American astronomers Smith and Terrile (97). Studies conducted by French astronomers over the past five years suggest that more than 100 comets fall on the central star each year (13). More recent estimates indicate that a total of 1000 comets per year, each a kilometer in size, is necessary to explain the observations of the disappearance of dusty cometary material into the central area of the disk where planets may be present (46). If the orbital system had planets like Earth, life might well be emerging now on one of them around the star Beta-Pictoris. A recent report has been made on the finding of a planetary system, with two Earth-like planets around a very distant neutron star, or pulsar in the Virgo constellation (101) and there is additional indirect evidence of the existence of planetary companions to nearby stars (17), and direct evidence for protoplanetary disks in the Orion Nebula (62).

Conclusions and reflections

As we have seen, the Universe is essentially reducing, but, rich in organic compounds and therefore conducive to the emergence of life, given the right conditions. But even more surprising, especially for those who study the nervous system, the brain, memory and other mental processes, is the realization that some of the simple molecules involved in the transmission of nervous impulses in living beings (neurotransmitters), such as glycine, glutamic acid and *gamma*–aminobutyric acid (GABA), have been found in the Murchison meteorite. One could therefore say that the Universe is not only prepared for the emergence of life, but also for the appearance of intelligence! This leads us to the intriguing corollary that the Universe might be populated by civilizations much more intelligent than those living on our small blue planet. Perhaps one day these advanced civilizations will be able to instruct us through interstellar communication as to the answers to human problems such as war, disease and old age. But until this utopian notion becomes a reality, we would do well to cherish our own small blue planet with all its varied and wonderful forms of life, the very ones that Darwin studied. After all, there is only one Earth in the Solar System.

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References

1. Altman, S. (1990). Ribonuclease postcript. J. Biol. Chem. **265**, 20053–20056.
2. Aller, L. H. (1961). The Abundance of the Elements. Interscience Publishers, New York, NY.
3. Awramik, S. M. (1981). The pre-phanerozoic biosphere—three billion years of crises and opportunities. *In* Nitecki, M. (ed.), Biotic Crisis in Ecological and Evolutionary Time, pp. 83–102. Academic Press, New York, NY.
4. Awramik, S. M. (1983). Filamentous fossil bacteria from the Archean of western Australia. Precambrian Res. **20**, 357–374.
5. Bada, J. L., Cronin, J. R., Ho, M.-S., Kvenvolden, K. A., Lawless, J. G., Miller, S. L., Oró, J., Steinberg, S. (1983). On the reported optical activity of amino acids in the Murchison meteorite. Nature **301**, 494–497.
6. Bada, J. L., Miller, S. L. (1987). Racemization and the origin of optically active organic compounds in living organisms. BioSystems **20**, 21–26.
7. Baeza, I., Ibanez, M., Lazcano, A., Santiago, C., Arguello, C., Wong, C., Oró, J. (1987). Liposomes with polyribonucleotides as model of precellular systems. Origins of Life **17**, 321–331.
8. Bailey, M. E., Clube, S. V. M., Napier, W. N. (1990). The Origin of Comets. Pergammon Press, Oxford, United Kingdom.
9. Baltscheffsky, H. (1993). Chemical origin and the early evolution of biological energy conversion. *In* Ponnamperuma, C., Chela-Flores, J. (ed.), Chemical Evolution: Origin of Life, pp. 13–23. A. Deepak Publishing, Hampton, VA.
10. Baltscheffsky, M., Baltscheffsky, H. (1992). Inorganic pyrophosphate and inorganic pyrophosphatases. *In* Ernster, L. (ed.), Molecular Mechanisms in Bioenergetics, pp. 331–348. Elsevier Sci. Publ. B. V., Amsterdam, Netherlands.
11. Baumann, U., Oró, J. (1993). Three stages in the evolution of the genetic code. BioSystems **29**, 133–141.
12. Bernal, J. D. (1949). The physical basis of life. Proc. Phys. Soc. A. **62**, 537–558.
13. Beust, H., Lagrange-Henri, A. M., Vidal-Majdar, A., Ferlet, R. (1990) The β Pictoris circumstellar disk. X. Numerical simulations of infalling evaporating bodies. Astron. Astrophys. **236**, 202–216.
14. Biemann, K., Oró, J., Toulmin III, P., Orgel, L. E., Nier, A. O., Anderson, D. M., Simmonds, P. G., Flory, P. G., Diaz, A. V., Rushneck, D. R., Biller, J. A. (1976). Search for organic and volatile inorganic compounds in two surface samples from the chryse planitia region of Mars. Science **194**, 72–76.
15. Bridson, P. K., Orgel, L. E. (1980). Catalysis of accurate poly(C)-directed synthesis of 3'-5'-linked oligoguanylates of Zn^{++}. J. Mol. Biol. **144**, 567–577.
16. Cameron, A. G. W., Benz, W. (1991). The origin of the Moon and the single impact hypothesis, IV. Icarus **92**, 204–216.
17. Campbell, B., Walker, G. A. H., Yang, S. (1988). A search for planetary mass companions to nearby stars. *In* Marx, G. (ed.), Bioastronomy. The Next Steps, pp. 83–90. Kluwer Acad. Publ., Dordrecht, Netherlands.
18. Cech, T. R. (1986). A model for the RNA-catalyzed replication of RNA. Proc. Natl. Acad. Sci. USA **83**, 4360–4363.
19. Cech, T. R. (1989). Ribozyme self-replication. Nature **339**, 507–508.
20. Cech, T. R. (1990). Self-splicing of group I introns. Annu. Rev. Biochem. **59**, 543–568.
21. Cronin, J. R. (1989). Origin of organic compounds in carbonaceous chondrites. Adv. Space Res. **9**, 54–64.
22. Cronin, J. R., Pizzarello, S. (1990). Aliphatic hydrocarbons of the Murchison meteorite. Geochim. Cosmochim. Acta **54**, 2859–2868.
23. Cronin, J. R., Pizzarello, S., Cruikshank, D. P. (1988). Organic matter in carbonaceous chondrites, planetary satellites, asteroids and comets. *In* Kerridge, J. F., Matthews, M. S. (ed.), Meteorites and the Early Solar System, pp. 819–857. University of Arizona Press, Tucson, AZ.

24. Chyba, C. F., Thomas, P. J., Brookshaw, L., Sagan, C. (1990). Cometary delivery of organic molecules to the early Earth. Science **249**, 366–373.
25. Darwin, C. (1859). On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London. (1964). A facsimile of the first edition. Harvard University Press, Cambridge, MA.
26. Deamer, D. W. (1986). Role of ampliphilic compounds in the evolution of membrane structure on the early Earth. Origins of Life **17**, 3–25.
27. Delsemme, A. H. (1991). Nature and history of the organic compounds in comets: an astrophysical view. *In* Newburn Jr., R. L. (ed.), Comets in the Post-Halley Era, vol. 1.
28. Delsemme, A. H. (1992). Cometary origin of carbon, nitrogen and water on the Earth. Origins of Life **21**, 279–298.
29. Doudna, J. A., Szostak, J. W. (1989). RNA-catalysed synthesis of complementary-strand RNA. Nature **339**, 519–522.
30. Epps, D. E., Sherwood, E., Eichberg, J., Oró, J. (1978). Cyanamide mediated synthesis under plausible primitive Earth conditions. V. The synthesis of phosphatidic acids. J. Mol. Evol. **11**, 279–292.
31. Fleischaker, G. R. (1990). Origins of life: an operational definition. Orig. Life Evol. Bios. **20**, 127–137.
32. Fox, S. W., Dose, K. (1977). Molecular Evolution and the Origin of Life. Marcel Dekker, New York, NY.
33. Gallant, R. A. (1994). Journey to Tunguska. Sky and Telescope **87**, 38–43.
34. Garrison, W. M., Morrison, D. C., Hamilton, J. G., Benson, A. A., Calvin, M. (1951). The reduction of carbon dioxide in aqueous solutions by ionizing radiation. Science **114**, 416–418.
35. Gilbert, W. (1986). The RNA world. Nature **319**, 618.
36. Haldane, J. B. S. (1929). The origin of life. Reprinted *In* Bernal, J. D. (1967), The Origin of Life, pp. 242–249. Weidenfeld and Nicolson, London, United Kingdom.
37. Harrington, J., LeBeau Jr., R. P., Backes, K. A., Dowling, T. E. (1994). Dynamic response of Jupiter's atmosphere to the impact of comet Shoemaker-Levy 9. Nature **368**, 525–527.
38. Hawker Jr., J. R., Oró, J. (1981). Cyanamide mediated synthesis of peptides containing histidine and hydrophobic amino acids. J. Mol. Evol. **17**, 285–294.
39. Ip, W. H., Fernandez, J. A. (1988). Exchange of condensed matter among the outer and terrestrial protoplanets and the effects on surface impact and atmospheric accretion. Icarus **74**, 47–61.
40. Irvine, W. (1989). Observational astrochemistry: recent results. Adv. Space Res. **9**, 3–12.
41. Joyce, G. F. (1991). The rise and fall of the RNA world. The New Biologist **3**, 399–407.
42. Kissel, J., Krueger, F. R. (1987). The organic component in dust from comet Halley as measured by the PUMA mass spectrometer on board Vega 1. Nature **326**, 755–760.
43. Kresak, L. (1978). The Tunguska object: a fragment of comet Encke? Bull. Astron. Inst. Czechosl. **29**, 129–134.
44. Krinov, E. L. (1960). Principles of Meteoritics. Pergamon Press, Oxford, United Kingdom.
45. Kvenvolden, K. A., Lawless, J. G., Perking, K., Peterson, E., Flores, J., Ponnamperuma, C. (1970). Evidence for extraterrestrial amino acids and hydrocarbons in the Murchison meteorite. Nature **228**, 923–926.
46. Lagage, P. O., Pantin, E. (1994). Dust depletion in the inner disk of β Pictoris as a possible indicator of planets. Nature **369**, 628–630.
47. Lazcano, A. (1994). The RNA world, its predecessors, and its descendants. *In* Bengtson, S. (ed.), Early Life on Earth: Nobel Symposium 84, pp. 70–80. Columbia University Press, New York, NY.
48. Lazcano, A. (1994). The transition from nonliving to living. *In* Bengtson, S. (ed.), Early Life on Earth: Nobel Symposium 84, pp. 60–69. Columbia University Press, New York, NY.
49. Lazcano, A., Guerrero, R., Margulis, L., Oró, J. (1988). The evolutionary transition from RNA to DNA in early cells. J. Mol. Evol. **27**, 365–376.
50. Leach, W. W., Nooner, D. W., Oró, J. (1978). Abiotic synthesis of fatty acids. *In* Noda, H. (ed.), Origins of Life: Proceedings of the 2 ISSOL and 5 ICOL Meeting, pp. 105–111. Center for Academic Publications, Japan Scientific Societies Press, Tokyo, Japan.
51. Levin, G. V., Straat, P. A. (1976). Viking labeled release biology experiment: interim results. Science **194**, 1322–1329.
52. Lowe, C. U., Rees, M. W., Markham, R. (1963). Synthesis of complex organic compounds from simple precursors: formation of amino acids, amino-acid polymers, fatty acids and purines from ammonia cyanide. Nature **199**, 219–222.

53. Miller, S. L. (1953). A production of amino acids under possible primitive Earth conditions. Science **117**, 528–529.
54. Miller, S. L. (1955). Production of some organic compounds under possible primitive Earth conditions. J. Am. Chem. Soc. **77**, 2351–2361.
55. Miller, S. L. (1957). The mechanism of synthesis of amino acids by electric discharges. Biochim. Biophys. Acta. **23**, 480–487.
56. Miller, S. L. (1977) The first laboratory synthesis of organic compounds under primitive Earth conditions. *In* Neyman, J. (ed.), The Heritage of Copernicus, pp. 228–241. MIT Press, Cambridge, MA.
57. Miller, S. L., Orgel, L. E. (1974).The Origins of Life on the Earth. Prentice Hall, Englewood Cliffs, NJ.
58. Miller, S. L., Urey, H. C., Oró, J. (1976). Origin of organic compounds on the primitive Earth and in meteorites. J. Mol. Evol. **9**, 59–72.
59. Müller, D., Pitsch, S., Kittaka, A., Wagner, E., Wintner, C. E., Eschenmoser, A. (1990). Chemie von α-aminonitrilen. Aldomerisierung von glycolaldehyd-phosphat zu recemischen hexose-2,4,6-triphosphaten und (in Gegenwart von Formaldehyd) racemischen pentose-2,4-diphosphaten: rac-Allose-2,4,6-triphosphat und rac-Ribose-2,4-diphosphat sind die reaktionshauptprodukte. Helv. Chim. Acta **73**, 1410–1468.
60. Noller, H. F., Hoffarth, V., Zimniak, L. (1992). Unusual resistance of peptidyl transferase to protein extraction procedures. Science **256**, 1416–1419.
61. Noone, D. W., Oró, J. (1979). Synthesis of fatty acids by a closed system Fischer-Tropsch process. *In* Kugler, E. L., Steffgen, F. W. (ed.), Hydrocarbon Synthesis from Carbon Monoxide and Hydrogen: Advances in Chemistry Series, vol. 178, pp. 159–171. American Chemical Society, Washington, DC.
62. O'Dell, C. R., Wen, Z., Hu, X. (1993). Discovery of new objects in the Orion Nebula on HST images: shocks, compact sources, and protoplanetary disks. Astrophys. J. **410**, 696-700.
63. Oparin, A. I. (1924). Proiskhodenie Zhizni. Moscoksky Rabotichii, Moscow. Translated as appendix *in* Bernal, J. D. (1967), The Origin of Life. World, Cleveland, OH.
64. Oparin, A. I. (1938) Origin of life. Macmillan Company, New York. (1953). An unabridged republication of the work published by the Macmillan Company. Dover Pub. Inc., New York, NY.
65. Oparin, A. I. (1957). The Origin of Life on Earth. Academic Press, New York, NY.
66. Orgel, L. E. (1987). Evolution of the genetic apparatus: a review. Cold Spring Harbor Symp. Quant. Biol. **52**, 9–16.
67. Orgel, L. E., Lohrmann, R. (1974). Prebiotic chemistry and nucleic acid replication. Acc. Chem. Res. **7**, 368–377.
68. Oró, J., Holzer, G. (1979). The photolytic degradation and oxidation of organic compounds under simulated martian conditions. *In* The Viking Mission and the Question of Life on Mars. J. Mol. Evol. **14**, 153–160.
69. Oró, J. (1960). Synthesis of adenine from ammonium cyanide. Bio. Chem. Biophys. Res. Commun. **2**, 407–412.
70. Oró, J. (1961). Comets and the formation of biochemical compounds on the primitive Earth. Nature **190**, 389–390.
71. Oró, J. (1961). Mechanism of synthesis of adenine from hydrogen cyanide under possible primitive Earth conditions. Nature **191**, 1193–1194.
72. Oró, J. (1963). Studies in experimental organic cosmochemistry. Ann. N. Y. Acad. Sci. **108**, 464–481.
73. Oró, J. (1965). Stages and mechanisms of prebiological organic synthesis. *In* Fox, S. W. (ed.), The Origins of Prebiological Systems, pp. 137–162. Academic Press, New York, NY.
74. Oró, J. (1988). Constraints imposed by cosmic evolution towards the development of life. *In* Marx, G. (ed.), Bioastronomy—The Next Steps, pp. 161–165. Kluwer Academic Publishers, Dordrecht, Netherlands.
75. Oró, J. (1994). Early chemical stages in the origin of life. *In* Bengston, S. (ed.), Early Life on Earth: Nobel Symposium 84. Columbia University Press, New York, NY.
76. Oró, J. (1994). Planetary formation of biochemical compounds in circumstellar habitable zones. *In* Shosthak, S. (ed.), Proceedings of the First International Conference on Circumstellar Habitable Zones. Icarus, in press.
77. Oró, J., Rappoport, D. A. (1959). Formate metabolism in animal tissues, II. The mechanism of formate oxidation. J. Biol. Chem. **234**, 1661–1665.
78. Oró, J., Holzer, G., Lazcano, A. (1980). The contribution of cometary volatiles to the primitive Earth. *In* Holmquist, R. (ed.), Cospar Life Sciences and Space Research, vol. 17, pp. 67–82. Pergamon Press, Oxford, United Kingdom.
79. Oró, J., Kimball, A. P. (1961). Synthesis of purines under primitive Earth conditions. I. Adenine from hydrogen cyanide. Arch. Biochem. Biophys. **94**, 217–227.

80. Oró, J., Kimball, A. P. (1962). Synthesis of purines under possible primitive Earth conditions. II. Purine intermediates from hydrogen cyanide. Arch. Biochem. Biophys. **96**, 293–313.
81. Oró, J., Miller, S. L., Lazcano, A. (1990) The origin and early evolution of life on Earth. Annu. Rev. Earth Planet Sci. **18**, 317–356.
82. Oró, J., Mills, T. (1989). Chemical evolution of primitive Solar System bodies. Adv. Space Res. **9**, 105–120.
83. Oró, J., Mills, T., Lazcano, A. (1992). Comets and the formation of biochemical compounds on the primitive Earth-A review. Orig. Life Evol. Bios. **21**, 267–277.
84. Oró, J., Rewers, K., Odom, D. (1982). Criteria for the emergence and evolution of life in the Solar System. Origins of Life **12**, 285–305.
85. Oró, J., Sherwood, E., Eichberg, J., Epps, D. E. (1978). Formation of the phospholipids under primitive Earth conditions and the role of membranes in prebiological evolution. *In* Deamer, D. (ed.), Light Transducing Membranes, pp. 1–19. Academic Press, New York, NY.
86. Owen, T., Gautier, D. (1989). Titan: some new results. Adv. Space Res. **9**, 73–78.
87. Peltzer, E. T., Bada, J. L., Schlesinger, G., Miller, S. L. (1984). The chemical conditions on the parent body of the Murchison meteorite: some conclusions based on amino, hydroxy and dicarboxylic acids. Adv. Space Res. **4**, 69–74.
88. Piccirilli, J. A., McConnell, T. S., Zaug, A. J., Noller, H. F., Cech, T. R. (1992). Aminoacyl esterase activity of the *Tetrahymena* ribozyme. Science **256**, 1420–1424.
89. Ponnamperuma, C. (1965). Abiological synthesis of some nucleic acid constituents. *In* Fox, S. W. (ed.), The Origins of Prebiological Systems and of their Molecular Matrices, pp. 221–242. Academic Press, New York, NY.
90. Sagan, C., Thompson, W. R., Khare, B. N. (1992). Titan: a laboratory for prebiological organic chemistry. Accounts of Chemical Research **25**, 286–292.
91. Schopf, J. W., Klein, C. (ed.) (1992).The Proterozoic Biosphere. Cambridge University Press, Cambridge, MA.
92. Schwartz, A. W., Orgel, L. E. (1985). Template directed synthesis of novel, nucleic acid-like structures. Science **228**, 585–587.
93. Shapiro, R. (1988). Prebiotic ribose synthesis: a critical analysis. Origins of Life **18**, 71–85.
94. Shen, C., Mills, T., Oró, J. (1990). Prebiotic synthesis of histidyl-histidine. J. Mol. Evol. **31**, 175–179.
95. Sherwood, E., Joshi, A., Oró, J. (1977). Cyanamide mediated synthesis under plausible primitive Earth conditions. II. The polymerization of deoxythymidine 5'-triphosphate. J. Mol. Evol. **10**, 193–210.
96. Sherwood, E., Nooner, D. W., Eichberg, J., Epps, D. E., Oró, J. (1978). Prebiotic condensation reactions using cyanamide. *In* Noda, H. (ed.), Origens of Life: Proceedings of 2nd ISSOL and 5th ICOL Meeting, pp. 113–122. Center for Academic Publications, Japan Scientific Societies Press, Tokyo, Japan.
97. Smith, B. A., Terrile, R. J. (1984). A circumstellar disk around b Pictoris. Science **226**, 1421–1424.
98. Urey, H. C. (1952) On the early chemical history of the Earth and the origin of life. Proc. Natl. Acad. Sci. USA **38**, 351–363.
99. Wakamatsu, H., Yamada, Y., Saito, T., Kumashiro, I., Takenishi, T. (1966). Synthesis of adenine by oligomerization of hydrogen cyanide. J. Org. Chem. **31**, 2035–2036.
100. Wilson, T. H., Maloney, P. C. (1976). Speculations on the evolution of ion transport mechanisms. Fed. Amer. Soc. Exp. Biol. Proc. **35**, 2174–2179.
101. Wolszczan, A. (1994). Confirmation of Earth-mass planets orbiting the millisecond Pulsar PSR B1257+ 12. Science **264**, 538–542.
102. Zahnle, K., Dones, L. (1992). Impact origin of Titan's atmosphere. Proc. Symp. on Titan, ESA SP-338, pp. 19–25.

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Prebiotic synthesis of organic compounds. A short review and new results

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Summary

Life on Earth is likely to have arisen in ten million years or less in a cold or temperate environment. This idea is based on the fact that the decomposition rates of organic compounds would not have allowed these prebiotic molecules to accumulate, particularly at high temperatures. Synthesis experiments using an electric discharge in a CH_4-containing reducing atmosphere have produced all but three protein amino acids. Fewer amino acids were produced in a non-reducing CO_2-containing atmosphere. Purines can be produced through mixtures of ammonium cyanide, while pyrimidine can be made from cyanoacetylene produced in electric discharge reactions of CH_4 and N_2. Sugars can be synthesized under alkaline conditions but ribose is not a major product, suggesting that ribonucleotides could not have been the first components of prebiotic genetic polymers. Plausible scenarios for the polymerization of amino acids to make peptides or the coupling of ribose to purines and pyrimidines to make ribonucleosides remain among the majorproblems in the origin of life studies.

Key words: origin of life, prebiotic chemistry, prebiotic atmosphere, nucleosides, 2'–5' bonds

Resumen

Es probable que la vida sobre la Tierra apareciera en el plazo de unos diez millones de años, o menos, en un ambiente frío o templado. Esta idea se basa en el hecho de que las tasas de descomposición de los compuestos orgánicos no hubieran permitido que se acumulasen esas moléculas prebióticas a altas temperaturas. Los experimentos de síntesis en los que se utiliza una descarga eléctricas en una atmósfera

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reductora con CH_4 han dado lugar prácticamente a todos los aminoácidos proteínicos menos tres. En una atmósfera no reductora con CO_2 se han producido menos aminoácidos. Las purinas se pueden originar a partir de mezclas de cianuro amónico, mientras que las pirimidinas se pueden producir mediante descargas en mezclas de CH_4 y N_2. Los azúcares pueden ser sintetizados en condiciones alcalinas pero la ribosa no es un producto importante, lo cual sugiere que los ribonucleótidos no habrían sido los componentes del material genético primitivo. El conocimiento de las condiciones en las que se dieron la polimerización de aminoácidos para producir péptidos, o el acoplamiento de la ribosa a las purinas y pirimidinas para hacer ribonucleósidos, continúan siendo uno de los grandes enigmas en los estudios del origen de la vida.

Introduction

Life arose on the Earth early in its history. The sequence of events started with the synthesis of simple organic compounds. These reacted to form polymers, which reacted to form structures of greater complexity (2, 3, 17, 33, 49). This is sometimes referred to as either the Oparin-Haldane or the heterotrophic hypothesis. Older ideas no longer regarded seriously include panspermia, spontaneous generation, and the hypothesis that the first organism arose by a very improbable event.

Oparin proposed that the first organisms were heterotrophic so they did not have to synthesize de novo the amino acids, purines, pyrimidines, and sugars to build proteins, nucleic acids, and other complex organic compounds within their cells. He proposed that the early Earth had a reducing atmosphere composed of methane (CH_4), ammonia (NH_3), water vapor (H_2O), and molecular hydrogen (H_2). Amino acids can be synthesized in surprisingly high yield from the action of sparks, simulating lightning discharges, on just such a strongly reducing atmosphere (28, 29, 30). For reviews dealing with the prebiotic synthesis of organic compounds and polymers (19, 31, 32).

Geological considerations

The time available for the origin of life. The Earth is 4550 million years (Ma) old, and the earliest fossils known, the Warrawoona microfossils and stromatolites, are about 3500 Ma in age. The time available for life to arise was probably much shorter than one billion years if the Earth melted completely early in its history or if the early Earth was heated as a result of the impacts of large asteroids.

Melting of the primitive Earth. At one extreme, it is believed that the condensation of cosmic dust took place in less than 100,000 years releasing gravitational energy that melted the entire Earth. At the other extreme, it is believed that the gravitational energy released during accumulation was dissipated by radiation so the crust would not have melted.

If the entire Earth did melt, all organic compounds would have been heated, pyrolyzed and completely destroyed. When the Earth had cooled sufficiently, a crust would have formed. Later,

organic compounds would have been synthesized and accumulated. It is possible that some of the organic compounds synthesized in the solar nebula were brought to the Earth with dust particles, meteorites, and comets. For unstable compounds, such as sugars, to accumulate, a continuous synthesis is required. Such compounds must then have been synthesized in the atmosphere or oceans of the primitive Earth.

It has been realized that because the Moon was bombarded by large objects until about 3800 Ma ago and so, the Earth must have been similarly bombarded. Large asteroids (50 to 600 km in diameter) are very destructive. A 600 km object would supply enough energy to boil the ocean and kill all but the hardiest thermophilic bacteria. Thus, any living systems that may have originated very early in Earth history could not have persisted until the end of the major bombardment, and prebiotic organic compounds also could not have accumulated until then (26, 47).

There are a number of reasons to think that life must have arisen in ten million years or less (22), based on the known rate of decomposition of organic compounds. The half-lives for decomposition at 25°C vary from several billion years for alanine, to a few million years for serine, to a few hundred years for sugars. The half-lives would be much less at temperatures of 50° or 100°C and longer at 0°C.

The temperature of the primitive ocean is not known, but it can be said that the instability of various organic compounds and polymers suggests strongly that life could not have arisen in the ocean unless the temperature was below 25°C. Another reason for believing that life originated at low temperatures is that all the template-directed reactions take place only below melting temperature of the appropriately organized polynucleotide structure (ranging from 0°C to 35°C). Since, the temperature coefficients of the synthetic reactions are generally less than those of the decomposition reactions, low temperatures would have favored the synthesis of more complex organic compounds and polymers.

The composition of the primitive atmosphere. Molecular oxygen (O_2) is usually assumed to have been absent from the primitive atmosphere (photodissociation of water vapor in the upper atmosphere is not a large source of O_2, and the oxygen formed would have reacted with Fe^{2+}, other unoxidized inorganic reactants or with organic compounds) during the period when organic compounds were synthesized up to the time when the first organism evolved. There is no geological evidence concerning the conditions on the Earth near the time of its formation, 4550 Ma ago. Even the 3800 Ma-old Isua rocks in Greenland are not sufficiently well preserved to provide much evidence about the atmosphere at that time. However, the more reducing atmospheres are more favorable for the synthesis of organic compounds, both in terms of the amount of yield and of the variety of compounds obtained.

Energy sources

No single source of energy nor any single process is likely to account for all the organic compounds on the primitive Earth. Ultraviolet light was probably the largest source of energy on the primitive Earth. Whether ultraviolet light was the most effective energy source for production of organic compounds is not clear. The yield of amino acids from the photolysis of CH_4, NH_3, and H_2O at wavelengths between 1470 and 1294 Å is quite low (16), probably due to the low yields of hydrogen cyanideThis is an

intermediate compound in the production of amino acids and most importantly for the prebiotic synthesis of the purines adenine and guanine. The most widely used sources of energy for laboratory synthesis of prebiotic compounds are electric discharges because they are very efficient in synthesizing hydrogen cyanide and easy to work with, whereas ultraviolet light is not except for the very short wavelengths.

Chemical components of living organisms

Before discussing the results of various types of prebiotic synthesis, we need to review the types of compounds in present-day living organisms. The chemical reactions within cells are almost all catalyzed by enzymes that are proteins. These proteins are made up of twenty different amino acids. The genetic information in a cell is contained in the nucleic acid DNA encoded by a sequence of four nucleotides (composed of one of the four nitrogen-containing bases, the sugar deoxyribose, and a phosphate). This information is transcribed to RNA. RNA differs from DNA by usually being single-stranded, and by containing the pyrimidine uracil in place of thymine, and the sugar ribose in place of deoxyribose. The RNA with the instructions for the synthesis of a protein enters the ribosome, where these instructions are used for the assembly of a protein.

A crucial problem for origin of life studies is to establish whether the first organism contained proteins, or RNA, or both. In favor of "proteins first" is the fact that amino acids are formed prebiotically and are polymerized more easily than RNA. In favor of "RNA first" is the fact that RNA can carry genetic information, but proteins cannot. For the present, the protein-RNA dilemma has been resolved in favor of RNA—by the discovery of ribozymes (4, 14, 15), that is RNA with catalytic activity. Still, it is necessary to always keep an open mind because so little is known about prebiotic events.

Prebiotic synthesis of organic compounds

Amino acids: synthesis in strongly reducing atmospheres. Mixtures of CH_4, NH_3, and H_2O, with or without added hydrogen (H_2), are considered strongly reducing atmospheres. The first successful prebiotic amino acid synthesis was carried out using an electric discharge as the energy source and a strongly reducing atmosphere (28, 29). The result was a large yield of amino acids together with hydroxy acids, short aliphatic acids, and urea. The products were not a random mixture of organic compounds; rather, a relatively small number of compounds, with few exceptions, of biological importance were produced.

Amino acids were not formed directly in the electric discharge, but were the result of solution reactions of smaller molecules produced in the discharge, in particular, reactions of hydrogen cyanide and aldehydes. Amino and hydroxy acids can be synthesized at high dilutions of HCN and aldehydes in a simulated primitive ocean. At least a small amount of atmospheric NH_3 would seem necessary for amino acid synthesis. Ammonia would have been decomposed in the early environment by ultraviolet light, but mechanisms for its resynthesis are also known.

In a typical electric discharge experiment, the CH_4 partial pressure of 0.1 to 0.2 atm is used for convenience. It is likely (but has never been demonstrated) that organic compound synthesis would work at much lower partial pressures of methane. There are no estimates available for pCH_4 on the primitive Earth, but low levels (10^{-5} to 10^{-3} atm) seem plausible. Higher pressures are not reasonable because the sources of energy would convert the CH_4 to organic compounds too rapidly for higher pressures of CH_4 to build up.

Ultraviolet light is not efficient in producing amino acids. Heating reactions (pyrolysis) of CH_4, and NH_3 give very low yields of amino acids. However, the pyrolysis of CH_4 and other hydrocarbons gives good yields of benzene, phenylacetylene, and many other hydrocarbons. It can be shown that phenylacetylene would be converted to the amino acids phenylalanine and tyrosine in the primitive ocean. Pyrolysis of hydrocarbons in the presence of NH_3 gives substantial yields of indole which can be converted to the amino acid tryptophan in the primitive ocean.

Because NH_3 would have dissolved in the ocean, a mixture of CH_4, N_2, H_2O, and traces of NH_3 is a more realistic atmosphere for the primitive Earth. The yields are somewhat lower than with higher partial pressures of NH_3, but the products are more diverse. Hydroxy acids, short aliphatic acids, and dicarboxylic acids are produced along with the amino acids. Thirteen of the twenty amino acids occurring in modern proteins can be formed in this single experiment (39, 50). Only the three basic amino acids, lysine, arginine, and histidine have not been synthesized under prebiotic conditions.

Amino acids: synthesis in mildly reducing and non reducing atmospheres. There has been less experimental work with gas mixtures containing CO and CO_2 as carbon sources instead of CH_4. Spark discharges have been the source of energy most extensively investigated. Fig. 1 compares amino acid

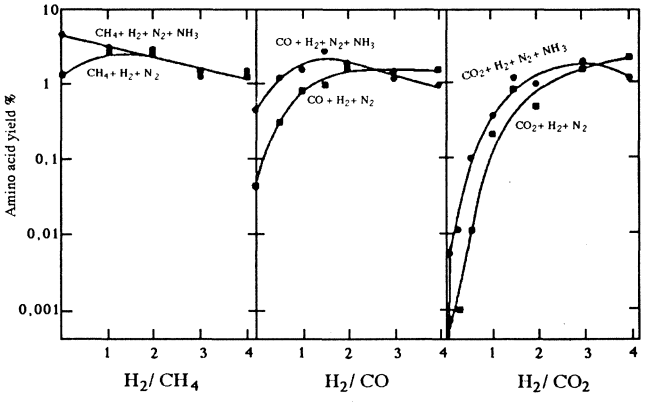


FIG. 1. Amino acid yields based on initial carbon. In all experiments pN_2 was 100 torr and pCH_4, pCO, or pCO_2 was 100 torr. The flask contained 100 ml H_2O for the curves with N_2 but no NH_3, and it contained 100 ml of 0.05 M NH_4Cl for the curves with N_2 + NH_3 (0.2 torr). The flask was kept at room temperature, and the spark generator was operated continuously for 48 h (from ref. 31).

yields using CH_4, CO or CO_2 as a carbon source in the presence of various amounts of H_2. CH_4 is the best source of amino acids using a spark discharge, but CO and CO_2 are almost as good if a high H_2/C ratio is used. With CO and CO_2, glycine was the predominant amino acid with little else besides some alanine being produced (44, 45, 48).

Other amino acids would probably have been formed from this glycine, H_2CO (formaldehyde), and HCN as the primitive ocean matured. CO and CO_2 are less favorable than CH_4 for amino acid synthesis. The synthesis of purines and sugars we describe later would not be greatly different with CH_4, CO or CO_2 as long as sufficient H_2 was available. It is not clear how such high molecular hydrogen to carbon ratios (H_2/CO >1 and H_2/CO_2 >2) could have been maintained in the primitive atmosphere, since H_2 escapes gravitationally from the Earth's atmosphere into outer space.

Purines and pyrimidines. If concentrated solutions of ammonium cyanide are refluxed for a few days, adenine is obtained in up to 0.5% yield along with 4-aminoimidazole-5-carboxamide and the usual cyanide polymer (35, 36). The difficult step in the synthesis of adenine is the reaction of the HCN tetramer with formamidine. However, this can be done using freezing solutions of HCN (42, 43). Guanine and the additional purines, hypoxanthine, xanthine, and diaminopurine could have been synthesized in the primitive environment by variations of the adenine synthesis, using aminoimidazole carbonitrile and aminoimidazole carboxamide.

The prebiotic synthesis of the pyrimidines of nucleic acids involves cyanoacetylene, which is synthesized in good yield by sparking mixtures of CH_4 and N_2. The cytosine, in turn, can be converted to uracil (8, 41). An alternative prebiotic synthesis of the pyrimidines cytosine and uracil starts with cyanoacetaldehyde which reacts with guanidine to give diaminopyrimidine (9, 10). This is hydrolyzed to cytosine and uracil.

Sugars. The synthesis of sugars from formaldehyde under alkaline conditions was discovered long ago. The "formose reaction" depends on the presence of a suitable inorganic catalyst [$Ca(OH)_2$ or $CaCO_3$, being the most commonly used] (1, 7, 13, 38). The reaction is autocatalytic and proceeds in a series of stages through glycolaldehyde, glyceraldehyde and dihydroxyacetone, four-carbon sugars, and five-carbon sugars to give finally hexoses, including the biologically important sugars glucose and fructose.

There are two problems with this reaction. The first is the instability of sugars (they decompose in a few hundred years or less at 25°C). One way of stabilizing sugars is to convert them into glycosides. The second problem is that the formose reaction gives a wide variety of sugars. Ribose occurs in the mixture, but it is not particularly abundant. It therefore has become apparent that ribonucleotides could not have been the first components of prebiotic nucleic acids (46). There are many possible substitutes for ribose, but the prebiotic synthesis of these has not been demonstrated.

In addition to the foregoing, numerous other compounds have been synthesized under primitive Earth conditions, including the following: dicarboxylic, tricarboxylic and fatty (C_2–C_{10}) acids, fatty alcohols, nicotinonitrile, nicotinamide. Other prebiotic compounds that may have been involved in polymerization reactions include the following: cyanate, cyanamide, HCN tetramer, phosphate polymers. A definitive statement cannot be made about the production rates and the concentrations of compounds

in the primitive ocean because the atmospheric composition, ambient temperature, and ocean size are unknown. Nevertheless, quantitative estimates have been made with results that give a relatively concentrated soup (48).

In some syntheses, the conditions are so forced that they could not be expected to have occurred extensively on the primitive Earth. Some important biological compounds that do not yet have adequate prebiotic syntheses are the following: arginine, lysine, histidine, straight chain fatty acids, porphyrins, pyridoxal, thiamine, riboflavin, folic acid, lipoic acid and biotin. Plausible prebiotic syntheses may become available before too long for some of these compounds. In other cases, the compounds may not have been synthesized prebiotically because their occurrence in living systems is a result of intracellular biochemical evolution that occurred after the origin of life.

Extraterrestrial organic syntheses

Organic compounds are abundant in certain carbonaceous meteorites, in the atmospheres of Jupiter and Saturn as well as Titan, the largest satellite of Saturn, and in interstellar dust clouds. Thus synthesis of organic compounds, in the absence of life, occurs widely in the solar system and beyond. A group of meteorites called carbonaceous chondrites contain 0.5% to as much as 5% organic carbon. A carbonaceous chondrite fell in 28 September 1969 near Murchison, Australia. Numerous pieces of meteorite were picked up immediately, and use of the most reliable analytical methods revealed large amounts of amino acids. The first report identified seven amino acids (20) and a second report, eighteen (6, 21).

There is a striking similarity between the amino acids produced by electric discharge and those occurring in the Murchison meteorite (50). The close correspondence suggests that the amino acids in the meteorite were synthesized on the parent body of the meteorite by means of an electric discharge or some analogous processes.

Interstellar molecules. In the past twenty years a large number of organic molecules have been identified in interstellar dust clouds, mostly based on emission lines observed in the microwave region of the spectrum (27). The molecules identified include formaldehyde (H_2CO), hydrogen cyanide (HCN), acetaldehyde (C_2H_4O), and cyanoacetylene (HC_3N). These are important compounds in prebiotic synthesis, but it is generally felt that interstellar organic molecules played, at most, a minor role in the origin of life on Earth because they are not likely to have been greatly concentrated and because they are likely to have been destroyed by ultraviolet light or by pyrolysis during entry of the atmosphere.

However, the presence of so many molecules of prebiotic importance in interstellar space, combined with the fact that their synthesis must differ from that on the primitive Earth where the conditions were vastly different, indicates that some organic molecules are particularly easily synthesized in the absence of living systems. In other words, there appears to be a universal organic chemistry, one that is manifest in interstellar space, occurs in the atmospheres of the major planets of the solar system, and must also have occurred in the reducing atmosphere of the primitive Earth.

Impacts of comets and meteorites have also been important in the Earth's history (1, 5). These were much more frequent earlier than 3800 millions of years ago and may have influenced the timing of the

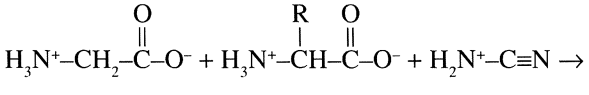
origin of life. How much organic material came in with the colliding objects, and what portion of it survived impact? Cosmic dust particles (<10 mm diameter) can survive impact because the atmosphere slows them down. Organic compounds in meteorites of less than a few meters diameter also survive impact, but larger objects are heated to a high temperature. Production of organic compounds on the primitive Earth under reducing conditions would far exceed the inputs from cosmic dust, meteorites, and comets. Only if conditions were nonreducing would extraterrestrial inputs of organic compounds be quantitatively important.

Polymerization processes on the primitive Earth

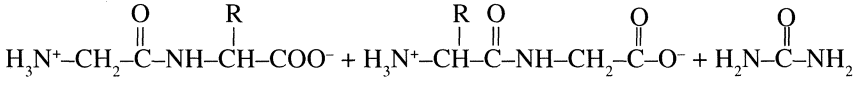
it has been established that numerous biologically important types of monomers can be synthesized under plausible prebiotic conditions and that similar syntheses have occurred both elsewhere in the solar system and in interstellar space. How might the prebiotic monomers have become chemically combined to form the type of polymers of which living systems are composed?

A large part of the energy produced by an organism is expended in polymerization of amino acids into proteins and of nucleotides into DNA and RNA (which are dehydration condensation reactions, thermodynamically unfavorable). In prebiotic processes this free energy barrier can be overcome in two ways: by coupling the removal of water to the hydration of a higher energy compound or by heating. Visible or UV light could in principle drive these reactions, but such processes are not known.

Peptide synthesis. The peptide bond, OC-NH, links amino acids into proteins. An example of the use of a high-energy compound to form a peptide bond is cyanamide, resulting in the linkage of glycine to leucine and formation of a dipeptide (37):



Glycine Leucine Cyanamide



Glycyl leucine Leucyl glycine Cyanamide + H_2O

The yields of this reaction are low. Use of ATP gives somewhat higher yields of dipeptides. Imidazole can be used as a catalyst. An alternate approach is to heat mixtures of amino acids at low relative humidities (11). The yields are good, but dry temperatures of 150° to 180°C do not occur extensively on the Earth. However the reaction goes very slowly at lower temperatures (40).

Prebiotic synthesis of nucleosides and nucleotides. The synthesis of nucleosides is one of the more difficult prebiotic polymerization reactions. Heating of ribose and purines at 100°C gives fair yields (2% to 20%) of the purine ribosides (12). However, heating a mixture of pyrimidines and ribose gives no detectable yield of nucleosides.

The phosphorylation of nucleosides to form nucleotides presents fewer problems (23). The first step is the synthesis of polyphosphates from ammonium dihydrogen phosphate. Under certain conditions, polyphosphates are produced in good yield:

$$n\ NH_4H_2PO_4 \xrightarrow[\text{excess urea}]{85°C \text{ to } 100°C} (NH_4PO_3)n + n\ H_2O$$

These polyphosphates can then phosphorylate nucleosides rather efficiently. If imidazole is present, imidazolides are produced which are similar in structure to ATP and are very reactive compounds that can be used for template polymerizations. Imidazole is a reasonable prebiotic compound but it is not clear whether large amounts of this molecule were present in the primitive ocean.

Template polymerization is the process in which polymerization of DNA is directed by the single strand of this nucleic acid acting as a template. The first version of this process must have taken place under reasonable geological (non-biochemical) conditions. The reaction of the activated purine ribotides on a polypyrimidine template proceeds quite efficiently, forming oligomers ranging from dimers up to molecules 50 monomeric units long or even longer (18, 24, 25, 34). Unfortunately, the complementary reaction of activated pyrimidines on a polypurine template does not work. It is possible that early template polymerizations used short oligomers. Otherwise, it is difficult to imagine that pyrimidines were involved in the earliest genetic system.

It is interesting to note that 2'–5' phosphodiester bonds between purine ribonucleotides are usually formed more easily than the desired 3'–5' bonds, bonds of the type occurring in the RNA (and DNA) or modern organisms. The 2'–5' bonds hydrolyzed more readily than 3'–5' bonds which, given sufficient time, would shift the population of polynucleotides in the primitve ocean over to those having the 3'–5' linkage, but it clearly would be better to obtain initially the 3'–5' isomers. Experimentally, 3'–5' isomers can be obtained directly by using a Zn^{2+} catalyst. The Zn^{2+}-catalyzed polymerization is only an order of magnitude of less fidelity than that exhibited by RNA polymerases occurring in present-day living systems.

These experimental template reactions are not efficient enough to replicate genetic information since pyrimidines do not react on a polypurine template. Furthermore, the imidazolides of the nucleotides are not realistically prebiotic, and the experiments were conducted using pure ribonucleosides as reagents, whereas prebiotic mixtures would contain purines and pyrimidines bonded to sugars other than ribose. It is clear that several pieces of this polymerization puzzle are missing. The only components of RNA that seem very primitive are the purines. It is a real challenge to discover the prebiotic replacements for the pyrimidines and ribose.

Concluding remarks

The environment of the primitive Earth is thought to have been more or less reducing; under these conditions, experimental studies have established that organic compounds would have been synthesized on a large scale. Many of the monomeric compounds thus produced are of importance in present-day organisms. These include amino acids, purines, pyrimidines, and sugars. Syntheses of precursors to such compounds take place at the present time in the atmospheres of Jupiter, Saturn, and Titan, as well as in interstellar space. Similar syntheses took place on the parent body (probably an asteroid) of the carbonaceous chondrites. To date, no highly efficient prebiotically plausible processes for the polymerization of amino acids to form peptides, or of mixtures of purines, pyrimidines, and ribose to form ribonucleosides, have been found. Considerable success has been obtained in the template polymerization of activated purines on polypyrimidine templates, but these reactions have not been accomplished under realistically prebiotic conditions.

Over recent years, investigations of the origin of life on Earth have been placed on a sound foundation. Much has been learned, but even more remains to be discovered. As a solvable scientific question of surpassing human interest, the problem of the origin of life stands out as one where the greatest advances are still to be made.

References

1. Anders, E. (1989). Pre-biotic organic matter from comets and asteroids. Nature **342**, 255–257.
2. Bada, J. L., Miller, S. L. (1968). Ammonium ion concentration in the primitive ocean. Science **159**, 423–425.
3. Bernal, J. D. (1951). The Physical Basis of Life. Routledge and Kegan Paul, London, United Kingdom.
4. Cech, T. R., Bass, B. L. (1986). Biological catalysis by RNA. Annu. Rev. Biochem. **55**, 599–629.
5. Chyba, C. F., Thomas, P. J., Brookshaw, L., Sagan, C. (1990). Cometary delivery of organic molecules to the early earth. Science **249**, 366–373.
6. Cronin, J. R., Moore, C. B. (1971). Amino acid analyses of the Murchinson, Murray, and Allende carbonaceous chondrites. Science **249**, 366–373.
7. Decker, P., Schweer, H., Pohlmann, R. (1982). Identification of formose sugars, presumable prebiotic metabolites, using capillary gas chromatogarphy/gas chromatography-mass spectrometry of *n*-butoxime trifluoroacetates on OV-225. J. Chromatogr. **225**, 281–291.
8. Ferris, J. P., Sanchez, R. A., Orgel, L. E. (1968). Studies in prebiotic synthesis. III. Synthesis of pyrimidines from cyanoacetylene and cyanate. J. Mol. Biol. **33**, 693–704.
9. Ferris, J. P., Zamek, O. S., Altbuch, A. M., Frieman, H. (1974). Chemical evolution, XVIII. Synthesis of pyrimidines from guanidine and cyanoacetaldehyde. J. Mol. Evol. **3**, 301–309.
10. Ferris, J. P., Chen, C. T. (1975). Chemical evolution. XXVI. Photochemistry of methane, nitrogen, and water mixtures as a model for the atmosphere of the primitive Earth. J. Am. Chem. Soc. **97**, 2962–2967.
11. Fox, S. W., Dose, K. (1972). Molecular Evolution and the Origin of Life. Marcel Dekker, New York, NY.
12. Fuller, W. D., Sanchez, R. A., Orgel, L. E. (1972). Synthesis of purine nucleosides. J. Mol. Biol. **67**, 25–33.
13. Gabel, N. W., Ponnamperuma, C. (1967). Model for origin of monosaccharides. Nature **216**, 453–455.
14. Gilbert, W. (1986). The RNA world. Nature **319**, 618.
15. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., Altman, S. (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell **35**, 849–857.

16. Groth, W., von Wayssenhoff, H. (1960). Photochemical formation of organic compounds from mixtures of simple gases. Planet Space Sci. **2**, 79–85.
17. Haldane, J. B. S. (1929). The origin of life. Rationalist Annual **148**, 3–10.
18. Joyce, G. F. (1987). Nonenzymatic template-directed synthesis of informational macromolecules. Cold Spring Harbor Symp. Quant. Biol. **52**, 41–51.
19. Kenyon, D. H., Steinman, G. (1969). Biochemical Predestination. McGraw-Hill, New York, NY.
20. Kvenvolden, K. A., Lawless, J. G., Pering, K., Peterson, E., Flores, J., Ponnamperuma, C., Kaplan, I. R., Moore, C. (1970). Evidence for extraterrestrial amino-acids and hydrocarbons in the Murchison meteorite. Nature **228**, 923–926.
21. Kvenvolden, K. A., Lawless, J. G., Ponnamperuma, C. (1971). Nonprotein amino acids in the Murchison meteorite. Proc. Natl. Acad. Sci. USA **68**, 486–490.
22. Lazcano, A., Miller, S. L. (1994). How long did it take for life to begin and evolve to cyanobacteria? J. Mol. Evol. **34**, 546–554.
23. Lohrmann, R., Orgel, L. E. (1973). Prebiotic activation processes. Nature **244**, 418–420.
24. Lohrmann, R., Orgel, L. E. (1976). Template-directed synthesis of high molecular weight polynucleotide analogues. Nature **261**, 342–344.
25. Lohrmann, R., Bridson, P. K., Orgel, L. E. (1980). Efficient metal-ion catalyzed template-directed oligonucleotide synthesis. Science **208**, 1464–1465.
26. Maher, K. A., Stevenson, D. J. (1988). Impact frustration of the origin of life. Nature **331**, 612–614.
27. Mann, A. P. C., Williams, D. A. (1980). A list of interstellar molecules. Nature **283**, 721–725.
28. Miller, S. L. (1953). Production of amino acids under possible primitive Earth conditions. Science **117**, 528–529.
29. Miller, S. L. (1955). Production of some organic compounds under possible Earth conditions. J. Am. Chem. Soc. **77**, 2351–2361.
30. Miller, S. L. (1957). The formation of organic compounds on the primitive Earth. Ann. NY. Acad. Sci. **69**, 260–274. [Also in: Oparin, A. (1959), The Origin of Life on Earth, pp. 123–135. Pergamon Press, Oxford.]
31. Miller, S. L. (1987). Which organic compounds could have occurred on the prebiotic Earth? Cold Spring Harbor Symp. Quant. Biol. **52**, 17–27.
32. Miller, S. L., Orgel, L. E. (1974). The Origins of Life on the Earth. Prentice-Hall, Englewood Cliffs, NJ.
33. Oparin, A. I. (1938). The Origin of Life. Macmillan, New York, NY.
34. Orgel, L. E. (1987). Evolution of the genetic apparatus: A review. Cold Spring Harbor Symp. Quant. Biol. **52**, 9–16.
35. Oro, J., Kimball, A. P. (1961). Synthesis of purines under primitive Earth conditions. I. Adenine from hydrogen cyanide. Arch. Biochem. Biophys. **94**, 221–227.
36. Oro, J., Kimball, A. P. (1962). Synthesis of purines under possible primitive earth conditions. II. Purine intermediates from hydrogen cyanide. Arch. Biochem. Biophys. **96**, 293–313.
37. Ponnamperuma, C., Peterson, E. (1965). Peptide synthesis from amino acids in aqueous solution. Science **147**, 1572–1574.
38. Reid, C., Orgel, L. E. (1967). Synthesis of sugar in potentially prebiotic conditions. Nature **216**, 455.
39. Ring, D., Wolman, Y., Friedman, N., Miller, S. L. (1972). Prebiotic synthesis of hydrophobic and protein amino acids. Proc. Natl. Acad. Sci. USA **69**, 765–768.
40. Rohlfing, D. L. (1976). Thermal polyamino acids: synthesis at less than 100°C. Science **193**, 68–70.
41. Sanchez, R. A., Ferris, J. P., Orgel, L. E. (1966). Cyanoacetylene in prebiotic synthesis. Science **154**, 784–786.
42. Sanchez, R. A., Ferris, J. P., Orgel, L. E. (1967). Studies in prebiotic synthesis. II. Synthesis of purine precursors and amino acids from aqueous hydrogen cyanide. J. Mol. Biol. **30**, 223–253.
43. Sanchez, R. A., Ferris, J. P., Orgel, L. E. (1968). Studies in prebiotic synthesis. IV. The conversion of 4-aminoimidazote-5-carbonitrile derivatives to purines. J. Mol. Biol. **38**, 121–128.

44. Schlesinger, G., Miller, S. L. (1983a). Prebiotic synthesis in atmospheres containing CH_4, CO, and CO_2. I. Amino acids. J. Mol. Evol. **19**, 376–382.
45. Schlesinger, G., Miller, S. L. (1983b). Prebiotic synthesis in atmospheres containing CH_4, CO, and CO_2. II. Hydrogen cyanide, formaldehyde and ammonia. J. Mol. Evol. **19**, 383–390.
46. Shapiro, R. (1988). Prebiotic ribose synthesis: a critical analysis. Orig. Life Evol. Bios. **18**, 71–85.
47. Sleep, N. H., Zahnle, K. J., Kasting, J. F., Morowitz, H. J. (1989). Annihilation of ecosystems by large asteroid impacts on the earth Earth. Nature **342**, 139–142.
48. Stribling, R., Miller, S. L. (1987). Energy yields for hydrogen cyanide and formaldehyde syntheses: the HCN and amino acid concentrations in the primitive ocean. Orig. Life Evol. Bios. **17**, 261–273.
49. Urey, H. C. (1952). The Planets. Yale University Press, New Haven, CT.
50. Wolman, Y., Haverland, W. J., Miller, S. L. (1972). Nonprotein amino acids from spark discharges and their comparison with the Murchison meteorite amino acids. Proc. Natl. Acad. Sci. USA **69**, 809–811.

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Life as a planetary phenomenon: the colonization of Mars

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Summary

Life is a planet-wide phenomenon in which its components incessantly move and interact. Life imperatively recycles its parts at the surface of the Earth in a chemical transformation and physical transport that depends utterly on the energy from a recent star, the Sun. Humanity, entirely dependent on other beings, plays a recent and relatively small part in the great phenomenon of life that transports and transforms the surface of the Earth. Our species accelerates but does not dominate the metabolism of the Earth system. Ironically, during the Apollo days of the sixties, fears were rampant that Martian or other extraterrestrial "germs" might "contaminate" our planet. After Viking, such fears are seen as the manifestation of cultural paranoia. The Viking missions complemented ground-based astronomical observation and yielded definitive evidence for the lack of life on the red planet. The Gaia hypothesis states that the surface temperature, composition of the reactive gases, oxidation state, alkalinity-acidity on today's Earth are kept homeorrhetically at values set by the sum of the activities of the current biota. Life, in other words, not only produces and maintains its immediate environment, but appears on Earth only as a planetary phenomenon. Since the natural tendency of all life is to grow exponentially to fill proximal volume, the question now "can life ecopoietically expand to Mars?" is entirely equivalent to the query of "can Gaia reproduce?"

Key words: terraforming Mars, ecopoiesis, Gaia, homeorrhesis, organic matter cycling

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Resumen

La vida es un fenómeno planetario, en el cual sus componentes se mueven e interaccionan incesantemente. La vida recicla imperativamente sus componentes en la superficie de la Tierra mediante transformaciones químicas y transporte físico, los cuales dependen completamente de la energía que proviene de una estrella reciente, el Sol. La humanidad, que depende en su totalidad de los otros seres vivos, desempeña un papel nuevo y relativamente pequeño en el gran fenómeno de la vida, cuya actividad principal es transportar y transformar la superficie de la Tierra. Nuestra especie ha acelerado el metabolismo del sistema Tierra, pero no lo ha dominado. Irónicamente, en los años de las misiones Apolo, en la década de los sesenta, se extendió el temor de que "gérmenes" marcianos, u otros "gérmenes" extraterrestres, pudiesen contaminar la Tierra. Después de las misiones de los Viking, se vio que esos temores era en realidad la manifestación de una paranoia cultural. Las misiones Viking complementaron observaciones astronómicas de la superficie de Marte y demostraron la ausencia de vida en el planeta rojo. La hipótesis Gaia afirma que la temperatura superficial, la composición de los gases reactivos, el estado de oxidación, la alcalinidad y la acidez de la Tierra actual se mantienen, por homeorresis, entre valores establecidos por el conjunto de actividades de la biota actual. En otras palabras, la vida no sólo produce y mantiene su ambiente inmediato, sino que se extiende por la superficie de la Tierra hasta convertirse en un fenómeno planetario. Dado que la tendencia natural de la vida es la de crecer exponencialmente para ocupar el volumen disponible en su entorno, la pregunta "¿puede la vida propagarse a Marte por ecopoyesis?" equivale a la pregunta "¿puede Gaia reproducirse?"

Introduction

We know very little about the origins of life. But we can say that the fossil record tells us that life appeared on Earth more than 3500 million years ago. And that a thousand million years or so later, in the late Archean or early Proterozoic eon, we recognize the presence in the fossil record of nucleated, or eukaryotic cells. These new kinds of organisms were not just simply large bacteria. They have a fundamentally different status. Eukaryotes are drawn on Carl Woese's trees as if "Eukarya" were equivalent to the lineages of prokaryotes. This is a systematic error in molecular phylogenetics (10). Cytoplasmic cell fusion, (e.g., in fertilization and in intracellular symbiogenesis) is one of several processes of eukaryotes that makes them fundamentally different from all bacteria. Eukaryotes have at least two sets of molecular machinery: two or more sets of DNA, of messenger RNAs, ribosomal RNAs, and so on. Eukaryotes are complex, products of fusion of two or more prokaryotes. They are chimeras. We suggest that prokaryotic organisms really do not speciate. Although we talk loosely about prokaryotic species, prokaryotes form a continuum. Genetic exchange is not blocked between prokaryotes. With the origin of eukaryotes, came the cessation of free genetic exchange. In our opinion, speciation itself occurred with eukaryosis: the acquisition of heterologous genomes to form the chimeric eukaryotes was exactly the process that originated speciation. Speciation resulted from cytoplasmic fusion and integration of heterologous genomes to irreversibly form, new individuals. Plotted on the geological time

scale, we have these big question marks: what is life?, and how life started? In the early evolution of bacteria a big split occurred to form two lineages: the eubacteria and the archaeobacteria. But both of these prokaryotic groups are single homogenomic systems; both are bacteria. All larger eukaryotic forms of life, plants, animals, fungi and protoctists, have an entirely different status from any bacteria. The eukaryotic forms are products of cell fusion events or symbiogenesis: the emergence of new organisms from protracted physical association between at least two kinds of predecessors.

Life as a planetary phenomenon

Product of the lively imagination of the British atmospheric chemist James E. Lovelock and the international space program, the Gaia idea has come of age. The atmospheric composition of Earth signals unmistakably that our planet is living: flanked by the dry, carbon dioxide-rich worlds of Mars and Venus, one invokes either physiological science or magic to explain Earth's wildly improbable, combustive, thoroughly drenched troposphere (Table 1).

Gaian environment regulation is achieved largely by the origin, exponential growth, and extinction of organisms, all related by ancestry and physically connected by proximity to the fluid phases (water and air) at Earth's surface (9). Organisms in communities form changing ecosystems that have persisted since the Archean. The interactions of organisms, driven by solar energy, produce and remove gases such that chemistry of non-noble gases, temperature, and alkalinity are actively maintained within limits tolerable to life.

TABLE 1. Some physical and chemical features of three planets of the Sun (at present)

	Venus	Earth	Mars
Diameter ($\times 10^3$ m)	12,104	12,756	6,794
Mass ($\times 10^{27}$ g)	4.8689	5.9742	0.64191
Density (g cm^{-3})	5.24	5.52	3.93
Mean dist. from Sun ($\times 10^9$ km)	108.2	149.6	227.9
Sidereal period (days)	224.7	365.3	686.9
Carbon dioxide (%)	98	0.03	95
Nitrogen (%)	1.7 (Ve)[a]	79	2.7 (Vi)[b]
Oxygen (%)	traces (Ve)	21	0.13 (Vi)
Methane (%)	none	0.0000015	none
Water (m)*	0.003	3000	0.00001
Pressure (atm)	90	1	0.0064
Temperature (K)	750	290	220

* Depth of water in meters over the planet if all vapor precipitated out of the atmosphere.

[a] Venera spaceship (USSR).

[b] Viking spaceship (USA).

Within this conceptual framework, biological as well as physical sciences become appropriate to the analysis of Earth's atmosphere and geologic history. Especially pertinent is the role of the microbiota (bacteria, protoctista, fungi) in Earth surface gaseous exchange, that involves the recycling of those chemical elements (e.g., H, C, O, N, P, S) absolutely required by life.

The Gaia hypothesis demonstrates how life sciences are essential to understanding Earth, while revealing the inadequacy of evolutionary theory developed in the absence of climatological and geological knowledge. The Gaian viewpoint is not popular because so many scientists, wishing to continue business as usual, are loath to venture outside of their respective disciplines. At least a generation or so may be required before an understanding of the Gaia hypothesis leads to appropriate, interdisciplinary research.

What is life?

Now, "fools walk in where angels fear to tread". So what is life? Living systems are bounded by membranes of their own making. They are not simply things; they are activities. Living beings are incessantly active. The essence of eukaryotes therefore is neither a sequence of nucleotides in the 18S RNA nor the membrane bounding the nucleus. The essence of eukaryotes is their multiple, composite genetic systems, the ability of the nucleated cells to interact in such a way as to not only fuse but to survive the fusion. In eukaryotes, the product of fusion actually survives such that two different, formerly independent cells with different genomes, merge to form a fundamentally new organism. The complex chimera, the product of cell fusion, is always eukaryotic. Neitheran archaeobacterium nor a eubacterium can merge its cells and survive in the complex state.

An example of this interaction occurs when the green alga *Trebouxia* and the fungus *Cladonia* grow together. A plant-like individual, a lichen that is neither the fungus nor the alga, is routinely produced. Indeed the lichen, a symbiogenetic complex, is a living being very different from either of its biotic components. This ability to fuse and form a fundamentally new individual is a eukaryotic property. Our speciation, an irreversible biological process that generates new organisms, began with eukaryosis, the formation of nucleated cells.

When two bits of fungal hyphae fuse to become one being, one does not digest the other, rather the fused product succeeds in growing. Indeed it grows into more complexity such that the mushroom we see is the product of the fusion. The product of the fusion is not just the single threads the parents were; rather fusion leads to the development of an entirely new organism. The difference between the sexual event of the fungal hyphal fusion and the symbiogenetic event of the fusion of the algal and fungal components of the lichen, is simply a consequence of the evolutionary distance that exists between the common ancestors of the individuals entering the merger. The two fungal threads that come together to form the mushroom have very recent common ancestors, whereas the alga and the fungus forming the lichen are very distant related. Speciation involves an irreversible evolutionary step, the formation of a new heterogenomic individual that must expend a great deal of energy to maintain its individuality. The essence of eukaryosis is actually thebehavior of permanent and reversible cell fusions.

Our phylogenetic trees drawn as if they can only branch, are systematically in error because in the evolution of all eukaryotes was the coming together of at least two phylogenies to form a third. The new

individual, formed by merger, is not the same as either of its phylogenetic or ontogenetic parents. Monera, or prokaryotes, were the products of the original origin-of-life event. These prokaryotic cells with their simple genomic prokaryotic cell organization, their small ribosomes and chromonemal DNA structure do not irreversibly speciate the way eukaryotes do. They have relatively naked DNA, no histone-DNA nucleosomal chromatin, only a single set of ribosomal RNAs, and many other differences relatives to any archaeobacterial or eubacterial prokaryotes. Of course diversification of prokaryotic ancestors led to the bacteria with which we are all now familiar: a group of microorganisms with great genetic and metabolic diversity. But we are grown up eukaryotes and pretend we have only one kind of ribosomes, ignoring the essence of the eukaryotic biology: we are the result of cell fusion events between prokaryotic ancestors.

Finally, if we take a look at geology, we can observe that minerals and living beings are not so separate as we might see them at a first glance. Many minerals are produced in and by life, sometimes in crystalline form. One of the most common minerals, calcium carbonate, is formed by marine organisms as shells. Another compound, calcium phosphate, is precipitated by the cells of the bones of vertebrates. As Table 2 shows, all five kingdoms of organisms have members which produce minerals. This list represents only a sample of the over fifty minerals now known to be produced by living cells (8).

Gaia as Earth's ecosystem physiology

When the Viking I and II missions to Mars returned their data, some members of the scientific community thought that "planetary biology" or "exobiology" were doomed because the absence of Martian life rendered them sciences with no object of study. Lovelock and his colleagues thought just the opposite: now that data from Mars were available, speculations comparing the planets could be replaced with knowledge. It became certain that the bleak Martian landscape is devoid of life, whereas life is not only a planet-wide phenomenon but in today's Solar System living beings are limited to Earth's biosphere.

Gaia has been called «Goddess of the Earth», or the «Earth as a single living being». These are misleading phrases. Since much scientific work mentioning Gaia suffers from problems of misunderstood terminology, we offer this physiologically oriented statement of the Gaia hypothesis.

The Gaia hypothesis states that the chemical composition of the reactive gases and the temperature of Earth's atmosphere are biologically controlled. Certain features, e.g., the salinity and alkalinity of the hydrosphere, are moderated by the biota (flora, fauna, and microbiota) in that their range of variation is kept within tolerable limits. Over 30 million types of live beings, descendants from common ancestors and members of five kingdoms, produce and remove gases, ions, and organic compounds. Their collective activity results in regulation of Earth's temperature and aspects of its surface composition: pH, oxidation state, etc. The chemical reactions of a physiology (unlike those of a strictly physicochemical system) are moderated by metabolism and growth (3). Without life, surface properties of Mars, Earth, and Venus would be extremely similar: abundant in carbon-dioxide with a small proportion of gaseous

TABLE 2. Some of the minerals produced by organisms*

Mineral	Bacteria	Protoctista	Plants	Fungi	Animals
CALCIUM					
Calcium carbonate ($CaCo_3$; aragonite, calcite, vaterite)	Sheath and other extracellular precipitate. *Achromatium.*	Amoeba and foraminiferan shells.	Extracellular precipitates.	Extracellular precipitates. Mushrooms.	Corals. Mollusk shells. Echinoderm skeletons. Calcareous sponges. Some kidney stones.
Calcium phosphate ($CaPO_4$)				Extracellular precipitates. Mushrooms.	Brachiopod "Lamp shells". Vertebrate teeth and bones. Some kidney stones.
Calcium oxalate (CaC_2O_4)			*Oxalis. Rumex. Dieffenbachia.*	*Penicillium, Aspergillus.*	Most kidney stones.
SILICON					
Silica (SiO_2)	Precipitates.	Diatom and radiolarian shells. Mastigote algae scales.	Grass phytoliths. Horse-tail stems.		Glass-sponge spicules.
IRON					
Magnetite (Fe_3O_4)	Magnetosomes.				Arthropods. Mollusks. Vertebrates.
Greigite (Fe_3S_4)	Magnetosomes.				
Siderite ($FeCO_3$)	Extracellular precipitates.				
Vivianite ($Fe_3[PO_4]_2 \cdot 8H_2O$)	Extracellular precipitates.				

Continued on following page

TABLE 2.—*Continued*

Mineral	Bacteria	Protoctista	Plants	Fungi	Animals
Goethite [α-FeO(OH)]	Extracellular precipitates.			Extracellular precipitates.	Chitons.
Lepidocrocite [γ–FeO(OH)]	Extracellular precipitates.			Extracellular precipitates. Mushrooms.	Chitons.
Ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$)			Flowering plants.		Mollusks.
MANGANESE					
Manganese dioxide (MnO_2)		Intracellular or extracellular precipitates around spores.			
BARIUM					
Barium sulfate ($BaSO_4$)		Algal-plastid gravity sensors. Marine protist skeletons.			Sense organs: statoliths (otoliths).
STRONTIUM					
Strontium sulfate		Marine protist shells.			Mollusk shells.

* Based on Table 2.1 of ref. 8.

nitrogen and very dry, reflecting their history, bulk composition, surface materials, proximity to the Sun, and interaction with solar radiation.

We reject the analogy that Gaia is a single organism, primarily because no single being feeds on its own waste nor, by itself, recycles its own food. Much more appropriate is the claim that Gaia is an interacting system the components of which are organisms. Nowhere is this more evident than in examples of biotic influence on important geological processes (Table 3) (14).

The two landers and orbiters of the 1975–1976 Viking missions to Mars yielded data that complemented earlier Earth-based observations of that planet. Organic compounds were absent: the concentration of total organics if present must be less than one part per billion. The gas-chromatographic detection of oxygen was not due to life but to the release of O_2 from moistened peroxides, and the incorporation of radioactive CO_2 was due to cosmic radiation, including UV photochemistry, and not to

TABLE 3. Biologically mediated significant geologic phenomena*

Example	Importance	Lithospheric reservoirs and examples
Phosphorus cycle	Component of DNA and RNA and ATP and NADPH nucleotides; phospholipid membranes and the calcium phosphate of bones. Because phosphate is a major growth-limiting nutrient, the P cycle is completely biologically mediated (2).	Earth's crust (inaccessible to life) and deep sea sediments; guano islands. Atmospheric phosphine (PH_3) is absent.
Calcium-carbonate deposition	Hard parts in shelled marine animals and many protoctists, i.e., foraminifera. Maintains pH balance in the ocean. As limestone is an important sink of CO_2. (See Table 2.)	Stromatolites. Coral reefs. Deep-sea carbonate sinking (foraminifera and coccoliths).
Organic matter deposition	Development of anoxic conditions and CH_4 production. As C is released to the atmosphere (preventing complete loss from the biosphere), elevated O_2 levels are maintained (13). Fossil fuels.	Oil shale and other organic-rich shales. Coal, peat, oil, tar sands.
Methanogenesis	Atmospheric composition of Earth (i.e., presence of methane, ozone) is inexplicable in the absence of life (13). (See Table 1.)	Trapped natural gas, swamp and marsh gas. Artthropod intestines. Vertebrated rumen.
Regolith consolidation	Consolidation of sediments by biotic communities, i.e., mucilage of microbial mats.	Mud. Unlithified sediment.
Erosion acceleration	Weathering rates increased by biologically mediated erosion, bacterial endoliths, fungal hypae, plant roots, and lichens.	Lithosphere-atmosphere-hydrosphere interfaces.
Microbially mediated mineral formation (biomineralization)	Genesis of important mineral deposits. Interpretation of modern and ancienet environments.	Banded iron formation. Witswatersrand gold deposits. Bog iron. Rock varnish. Manganese nodules.

* Based on Table 2 of ref. 11.

photosynthesis. Once the reactants were spent, no new change was detected by these experiments. The conclusion is inescapable: no evidence exists for present life on Mars. The same is true of Venus.

As far as we know, the Gaia phenomenon is limited to Earth. Can it be extended by colonization of Mars? Comparison of Earth with Mars helps highlight both the nature of Gaia and implications of the idea for the study of Earth (9).

The concept of ecopoiesis

The quest for life on Mars which began by telescope long before the Viking missions will not likely end with the deployment of rovers on the planet early in the next century. After acceptable confirmation that Mars is uninhabited, the next task might be to "seed" the red neighbor with propagules from Earth. (Many will justifiably argue that the resolution of more pressing Earth-based problems should be a far greater priority: curbing the human tendency to convert the surface of Earth to urban ecosystem or fostering and documenting the diversity of life).

The first and perhaps most crucial task in making Mars habitable is to increase its surface temperature. Proposals for heating Mars have ranged from engineering dreams of melting the ice caps with giant orbiting mirrors or covering the surface with black lichens, to schemes of rocketing greenhouse chlorofluorocarbons (CFCs) into the atmosphere. Recent proposals tend to be more detailed and slightly more feasible, yet share with their forerunners a profound, simultaneous strength and weakness: although such schemes are ambitious enough to excite the imagination, making captivating layouts in popular science magazines, they are too grandiose and vague to be practical.

For example, even if several millions of tons of new, UV-resistant CFCs could be produced annually in situ from the surface of Mars, leading to a release of carbon dioxide and to planetary temperatures of 22°C, then what? Even if oceans appeared from ice trapped in the lower latitudes because a way had been found to return to the atmosphere the CO_2 now trapped in surface carbonates, what now? The density (and therefore livability) of a Martian atmosphere is probably intrinsically limited by the weakness of Mars magnetic field. In the absence of magnetic deflection of solar wind a Martian atmosphere would quickly be ablated. Even if genetically engineered plants and microbes were created to produce oxygen and other gases at hitherto miraculous rates, it still could take, as Christopher McKay (NASA Ames Research Center) estimates, about a thousand years to build an atmosphere to stable levels of oxygen in carrier gases breathable by eukaryotic microbes, let alone humans (12, 13).

Although the new science of geophysiology and the success of biotechnology with microorganisms may have incited us to fantasies of planetary design, colonizing Mars so that humans might walk in the open along its canyons remains a distant fantasy. One should distinguish here between ecopoiesis (5, 6; the inundation of a formerly uninhabited surface with viable living systems) and terraformation (the recreation of Earth on another planetary surface). For the foreseeable future, ecopoiesis but not wholesale terraformation seems a possibility for Mars; the former is, however, a prerequisite for the latter (11). Ecopoiesis would not make Mars into an extraterrestrial paradise, so much as it would transform it into a global cesspool, colorful, perhaps, but rich in mephitic vapors. The early history of Earth, after all, and the present state of the gas giants in the outer Solar System are characterized by a chemistry that

more resembles sewer gas than food. Though alien and inhospitable to mammals, these reduced sulfurous carbon-rich volatile compounds were crucial to the origin and early evolution of life.

One way to make a planetary surface livable may be to repeat the evolutionary colonization process that occurred on Earth, which began with hydrogen, methane, ammonia, formaldehyde, sulfides, nitriles, and simple sugars. Shortly after life appeared, noxious gas exchanges among anoxygenic phototrophic bacteria and their dependents ensued. Navertheless, although the outcome of a rushed and deliberate Martian colonization process is likely to be highly unpredictable, possibly even tragic, it is pretty sure that a few human generations from now the experiment will take place.

Will we humans, Godlike, wave our magic wand? Do we really think, in our naiveté, that strewing our scientific instrumentation over the red surface of Mars via robots in a geological wink of an eye will produce a New Blue Earth? Far more probably, Mars will be colonized slowly and gradually, and not by humanity but through humanity, facilitated by robots. For the foreseeable future it seems likely that the only human presence on Mars will be via the developing technology of telepresence. The landing of the two remote-sensing, remote-controlled, human connected Viking landers in 1976 proves that the process of colonization has already begun. Unlike Neil Armstrong's epochal "one step for man, one giant leap for mankind", the ecopoiesis of Mars's surface has no instantly recognizable moment. The launch of human-built life detectors to Mars, the "telepresent" sensory cameras that radio their signals back to eager humans at mission control, space-crew first landings, early orbiting Mars stations, and the eventual habitation of the red surface by emigrants of a variety of species all are part of a gradual process of ecopoiesis. All are likely to occur haphazardly, with very little conscious planetary bioengineering.

The distinction between altering one's body to "adapt" to any inhospitable environment and altering the environment itself is largely specious from a Gaian viewpoint. As organisms evolve, both their bodies and the environment change irreversibly (4). Such change occurs through technology, which is not a uniquely human phenomenon. Animate and inanimate nonhuman technologies abound, e.g., wasp nests, humidified and air-conditioned termite mounds, or the immense lithified limestone reefs fringing tropical islands.

Propagules of life

Life packages its precious contents: production of heat-proof bacterial endospores, dinomastigote cysts, formation by trees of seeds and hardened fruits, rubbery eggs of snakes, or the tough eggcases of rays. Among the most remarkable of such propagules are the "tuns" of tardigrades or the salt-tolerant dust-like eggs of brine shrimp.

To enable any Earthlings to dwell on the surface of Mars, bubblelike enclosures probably will be required that house a complexity of species in self-supporting recycling systems, in principle like the stated goals of the exorbitant Biosphere II project in Arizona's Sonoran desert. This incipient Earth-propagule (which "germinated" and released its contents in September 1993) contained eight "biospherians". The 17-acre facility allegedly was "materially closed" in the autumn of September 1991 to all but its enormous intake of external electrical power. It is clear that at present we are far from establishing any such immodest biospheres or even their miniaturized counterparts on Mars. The energy

needed for the mere sustenance of any living system on the red planet, will require at least onsite nuclear power. However, as soon as adequately closed artificial biospheres are established e.g., to serve as base camps for CFC factories global, terrestrial, biospheric Earth life will have de facto, if inconspicuously, colonized the surface of Mars.

Such an artificial biosphere, a radiation and desiccation-resistant form, is highly reminiscent of large-scale nonhuman evolutionary innovations far more continuous with the past than it seems at first glance. By packaging and miniaturizing the essentials for survival, life ventures out upon and ultimately makes a home for itself in formerly hostile terrain.

The ecopoiesis of Mars would likely be accomplished by interaction of many types of Earth organisms: bacteria, protoctists (mainly as algae), plants and fungi will certainly play their roles. Indirectly, all life forms would be involved in planetary colonization, although at first multispecies bases will need to be constructed in an effort planned by exceedingly few highly select, and passionately dedicated humans. Such bases are necessary to protect their inhabitants from an initially hostile external Martian world. Food plants must be grown and all wastes internally recycled.

That such enclosures of metal, glass, and plastic might be built by scientists, engineers, and other working people is hardly an argument for their absolute uniqueness: all previous technological advances in the evolution of life (e.g., silica fretwork of diatoms, calcium phosphate bone and teeth in vertebrates, lignification leading to great height in plants, and the chitinous exoskeletons of insects and crustaceans) involved more than a single type of life and were prerequisite to the adaptive radiation of their inventors into new and formerly hazardous realms.

Humans by no means have an "exclusive" on technology. Magnetite teeth in molluscs and wax synthesis by hymenopterans are technologies that preceded those of *Homo sapiens* by millions of years. Calcium phosphate teeth, barium sulfate gravitational sensors, and temperature- and humidity-controlled termite mounds were as much a prerequisite for cosmopolitan Cenozoic distribution of, say, rodents, charalean algae, and fungi-gardening termites as telephones and electric power are to human urban expansion. Silurian-Devonian emigration of life to the land, with its attendant problems of lack of support by water, depleted nutritional substrates, and its exposure to continuous solar UV radiation, demanded a dramatic repackaging of life's resources an incorporation into bodies of what at one time could be found only "outside" in the mineral environment (14).

Such repackaging of living beings and their accoutrements might begin within recycling enclaves, "artificial biospheres". Above and beyond anything done later, the first of these bases on Martian terrain would already be colonization of Mars. Cosmic historians, in retrospect, might use establishment of such Martian base camps to date the reproduction of planetary life. Such "artificial biospheres" might be recognizable not merely as a human technology but as an expansion and metamorphosis of Earth's original biosphere by members of all of the five kingdoms of life. Gaia would have reproduced, challenging the objection of Doolittle (1) that Gaia cannot be a life form because it is incapable of reproduction. Seen from afar, the settling of Mars would be akin to budding, a space-borne planting of a "sporulated" form of biospheric life Gaia transporting propagules of itself to the surface of a new world.

A Gaian scientific world view is especially relevant in light of expansive human-wrought modification of the global environment and the talk about further missions to Mars. Although the fundamentals of Lovelock's Gaia hypothesis have not changed in 25 years, researchers still do not yet

understand them. The Gaian approach critically empowers research on Earth systems precluded by the patchiness of the "academic apartheid" from which Lovelock, as a young man, fled.

The Gaian concept of physiological surface regulation is unpalatable, especially to those who hold dogmatic ideas on Earth processes. Lovelock remarked that the Gaia hypothesis has not been controversial; it has just been ignored. But the scientific details (contained in the literature listed in the appendix of ref. 11), are becoming better known. We are hopeful that the full importance of the Gaia idea will continue to be more extensively understood by scientists and students, especially by geologists upon whom rest the future of Gaia-oriented scientific research.

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References

1. Doolittle, W. F. (1981). Is nature really motherly? CoEvolution Quarterly **29**, 58–63.
2. Filipelli, G. M., Delaney, M. L. (1992). Similar phosphorus fluxes in ancient phosphorite deposits and a modern phosphogenic environment. Geology **20**, 707–712.
3. Guerrero, R., Mas, J. (1989). Multilayered microbial communities in aquatic ecosystems: growth and loss factors. *In* Cohen, Y., Rosenberg, E. (ed.), Microbial Mats. Physiological Ecology of Benthic Microbial Communities, pp. 37–51. American Society for Microbiology, Washington, DC.
4. Guerrero, R., Mas-Castellà, J. (1995). The problem of excess and/or limitation of the habitat conditions: do natural assemblages exist? *In* Joint, J. (ed.), Molecular Ecology of Aquatic Microbes. pp. 191–204. NATO ASI Series G, 38. Springer-Verlag, Berlin, Germany.
5. Haynes, R. H. (1990). Ecce ecopoiesis: playing God on Mars. *In* MacNiven, D. (ed.), Moral Expertise: Studies in Practical and Professional Ethics, pp. 161–183. Routledge, London, United Kingdom.
6. Haynes, R. H. (1992). How might Mars become a home to humans? Gaia Science **2**, 7–9.
7. Lovelock, J. E., Margulis, L. (1974). Atmospheric homeostasis by and for the biosphere: the Gaia hypothesis. Tellus **26**, 2–10.
8. Lowenstam, H. A., Weiner, S. (1989). On Biomineralization. Oxford University Press, New York, NY.
9. Margulis, L, Guerrero, R. (1989). From planetary atmospheres to microbial communities: a stroll through space and time. In Botkin, D. B., Caswell, M. F., Estes, J. E., Orio, A. A. (ed.), Changing the global environment, pp. 51–67. Academic Press, New York, NY.
10. Margulis, L., Guerrero, R. (1991). Kingdoms in turmoil. New Scientist **129**, 46–50.
11. Margulis, L., West, O. (1993). Gaia and the colonization of Mars. GSA Today **3**, 277–291.
12. McKay, C. P. (1987). Terraforming: making an Earth of Mars. Planetary Report **7**, 26–27.
13. McKay, C. P., Toon, O. B., Kasting, J. F. (1991). Making Mars habitable. Nature **352**, 489–496.
14. Sagan, D. (1992). Metametazoa: biology and multiplicity. *In* Crary, J., Kwinter, S. (ed.), Incorporations (6. Zone), pp. 362–385. MIT Press, Cambridge, MA.

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Cellular evolution during the early Archean: what happened between the progenote and the cenancestor?

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Summary

Although cladistic techniques cannot be applied to the understanding of the origin of life itself, at the time being the comparison of macromolecules is not only the most powerful tool for inferring the branching order of the three cell lineages, but also for providing some insights into the nature of the biological systems that preceded their last common ancestor. It is argued that this information cannot be extrapolated to support the hypothesis that the first living systems were hyperthermophiles that emerged in deep sea vents or in other extreme environments. The significance of detecting and characterizing paralogous genes that duplicated before the divergence of the last common ancestor of all extant life to understand some of the mechanisms that led to the establishment of biochemical pathways is also discussed.

Key words: cellular evolution, Archean times, molecular cladistics, progenote, cenancestor

Resumen

A pesar de que las técnicas cladísticas no son por sí mismas aplicables al conocimiento del origen de la vida, en la actualidad la comparación de macromoléculas no es sólo la herramienta más potente para determinar el orden de divergencia de los tres linajes celulares, sino también para proporcionar algunas indicaciones sobre la naturaleza de los sistemas biológicos que precedieron a su antepasado común. Esta

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información no puede extrapolarse para fomentar la hipótesis de que los primeros sistemas vivos fueron hipertermófilos que surgieron en los "deep sea vents" o en otros ambientes extremos. En este artículo se discute también la importancia que tiene la detección y caracterización de genes parálogos, que se duplicaron antes de la divergencia del último antepasado común, para el conocimiento de algunos mecanismos que condujeron al establecimiento de rutas bioquímicas básicas.

Introduction

Although molecular techniques have been used in phylogenetic studies since the turn of the century (44), it was not until much latter that it was fully realized that protein and nucleic acid sequences are historical documents (73) that contain an extraordinarily rich amount of evolutionary information of unsurpassed value whose retrieval has led to several major conceptual revolutions in contemporary biology. However, it is also true that this approach has remarkable limitations. Several attempts have been made to extrapolate the results of macromolecular comparisons back into the stages in which the basic characteristics of living systems were first established (12, 60). Nevertheless, these endeavours have been hindered by our almost complete ignorance of the nature of the first living systems and thus they lack, as argued below, evolutionary significance. Indeed, it is becoming increasingly clear that, although the development of molecular phylogeny has greatly deepened our understanding of the early stages of cellular evolution, the complex—and perhaps unfathomable—issue of the origin of life is not amenable in itself to cladistic analysis.

Of course, the significance of molecular cladistics in the discovery of the three major cell lineages, i.e., the eubacteria, the archaeobacteria, and the eukaryotic nucleocytoplasm (69) cannot be underscored. As argued throughout this paper, the evolutionary analysis of the available databases can provide important insights not only on the nature of their last common ancestor, but also on the biological events that preceded it, during which some of the essential traits of basic metabolic pathways were shaped (71). Therefore, the purpose of this paper is to summarize our current knowledge of an early stage of cellular evolution that took place before the diversification events of the last common ancestor of all extant life forms, but after the appearance of biological systems, very likely of cellular nature, already endowed with a genetic code and a ribosome-mediated protein synthesis.

Progenotes, cenancestors and molecular cladistics

Although attempts to find the proper place of microbes in phylogenetic trees date back to the work of Ernst Haeckel and other 19th century scientists, it was not until much latter that the first detailed theories on prokaryotic evolution and classification where first suggested. On the basis of morphological features, Kluyver and van Niel (32) suggested that three different lines of descent formed by the spirilla, the sporulating Gram-positive bacilli, and the high actinobacteria, respectively, were descendants of primitive coccoidal bacteria. That same year A. I. Oparin, on the basis of a detailed comparison of the basic biochemical processes and energy-generating metabolic pathways, published the Russian edition

of his book on the origin of life, in which he suggested an evolutionary scheme that begun with anaerobic heterotrophy and proceeded, in a gradual, stepwise evolutionary process that eventually led to oxygen-producing photosynthetic cyanobacteria (45).

Although the points of view suggested by Oparin have an enormous heuristic value, which eventually led to the establishment of an entire field of scientific research devoted to the scientific study of the origin of life, it is no longer possible to assume that the first living system was a *Clostridium*-like, anaerobic fermenter (55) or a *Mycoplasma*-type of prokaryote (47, 67). The nature of the first living forms is still an open question, but as summarized by Woese (69), molecular cladistic studies have shown that simplicity is not equal to primitiveness: the wall-less aphragmobacteria phenotype is polyphyletic and has evolved independently both among the eubacteria and the archaeobacteria, while the anaerobic relatively simply clostridial lifestyle is part of the Gram-positive, low GC branch that is very far away from the oldest eubacterial phenotypes.

It is now generally accepted that the development of molecular cladistics has led to major changes in our current understanding of microbial evolution. A major achievement of this approach has been the use of small subunit ribosomal RNA (rRNA) sequences as phylogenetic markers. The advantages of using these phylogenetic markers are: (i) the universal distribution of their genes in all known organisms and organelles of cellular origin; (ii) the fact that they always serve the same function; (iii) their improbable lateral gene transfer; and (iv) the relatively slowly change in primary sequence compared to other molecular clocks and may be thus used for the construction of deep phylogenies (69).

It is frequently forgotten that the evolutionary comparison of 16S rRNA-like genes allows the construction of cladograms depicting the phylogeny of genes, but not of organisms. However, the properties of rRNA genes as phylogenetic markers make them valuable instruments, which have allowed the construction of a trifurcated, unrooted tree in which all known organisms can be grouped in one of the three major cell lineages: the eubacteria, the archaeobacteria, and the nucleocytoplasmic component of eukaryotes (69). Since in unrooted rRNA-based cladograms no single major branch predates the other two, and all three derive from a common ancestor, it was concluded that the latter corresponded to an ancestral form of life much simpler than contemporary prokaryotes, i.e., the progenote (70). This hypothetical entity was defined as a primitive system with a rudimentary translation machinery, in which phenotype and genotype still had an imprecise, rudimentary linkage relationship (70). A model for the progenote described it as endowed with a fragmented, disaggregated genome formed of double-stranded RNA genes, many of which could have existed in multiple copies (68). Independent evolutionary refinements along the three lines of descent not only led into its aggregation into DNA genomes, but led also to the differences found among the transcription and translation machineries of eubacteria, archaeobacteria and eukaryotes after their divergence from their last common ancestor (69).

No outgroups are known for rRNA based phylogenies, which specify branching relationships but not the position of the universal ancestor. Therefore, it is not possible to identify in them the oldest cellular phenotype. Nevertheless, speculations on the antiquity of a trait may be justified on empirical generalizations based both on the trait's essential role and on its wide distribution. Thus, a partial description of the last common ancestor of the three main branches may be infered from the distribution of homologous characters among its descendants, i.e., by comparing eubacteria, archaeobacteria and eukaryotes and see which monophyletic traits are common to the three of them. It can be argued that any feature found in all three lines was probably present in the ancestral organism from which they are

derived, i.e., that genes present in the main branches of the universal rRNA tree which are not the result of horizontal transfer must have been also present in their evolutionary progenitor.

As shown in Table 1, the set of such traits that have been identified as of 1994 is still small, but the picture of the last common ancestor that can be constructed is that of a rather sophisticated cell. As suggested by the presence of genes involved in major anabolic processes that include the biosynthesis of purines, pyrimidines, coenzymes, arginine, tryptophan, histidine and the branched-chained amino acids, i.e., valine, isoleucine, and leucine (Table 1), its biosynthetic abilities were comparable to those of modern cells. The occurrence of insulin-like peptides and cAMP in the three cell lines suggests that signalling molecules involved in intracellular and cell-to-cell communication had already appeared in their last common ancestor, as well as heat-shock proteins and other molecules that may have been involved in responses to environmental insults and stress conditions.

TABLE 1. Homologous traits common to the three cellular domains[a]

(i) Traits involved in replication and protein biosynthesis	
DNA polymerase B*	elongation factor 1α/Tu*
Gyrase B	elongation factor G/2*
DNA topoisomerase II	isoleucyl-tRNA synthetase*
RNA polymerases*	ribonuclease P
polynucleotide phosphorylase	ribosomal proteins S9, S10, S17, S15, L2, L3, L6, L10, L11, L22, and L23
(ii) Traits involved in energy generation processes and in biosynthetic pathways	
F-type ATPase α subunit*	arginosuccinate synthetase
F-type ATPase β subunit*	aspartate aminotransferase*
carbamoyl-phosphate synthetase*	cytrate synthetase
glucose 6-phosphate dehydrogenase	enolase
glutamate dehydrogenase II*	glutamine synthetase
malate dehydrogenase*	phosphoglycerate kinase
pyruvate: ferrodoxin oxidoreductase	porphobilinogen synthase
histidinol phosphate aminotransferase	purine biosynthetic genes
tryptophan biosynthetic genes	branched-chain amino acid biosynthetic genes
(iii) Traits involved in environmental response and chemical signalling	
cAMP	
insulin-like polypeptides	
heat shock protein 70	
Mn/Fe superoxide dismutases*	
photolyases	

[a] Based on Lazcano et al. (38), Benner et al. (7), and Doolittle and Brown (13).

* Paralogous duplicate.

The structural similarities shared by the proteins found in all three lines of descent suggest that considerable fidelity already existed on the then operative genetic system of their last common ancestor, which might have been already based on double-stranded DNA molecules (38). This conclusion is supported by the analysis of the available sequence databases, as well as by information derived from antibiotic sensitivity and antibody response, which suggests that the ancestor of the three lines already encoded oligomeric RNA polymerases, DNA topoisomerases, DNA polymerases with proof-reading activity (17), and photolyases involved in the monomerization of UV-pyrimidine dimers (62). Thus, it was already endowed with mechanisms assuring both high-fidelity replication and repair of UV-induced DNA damage, which would be extremely valuable in the anoxic primitive environment.

Although the presence of superoxide dismutase in *Methanobacterium thermoautotrophicum* (63), and of cytochrome oxidase in *Sulfolobus acidocaldarius* (8) raises the troublesome possibility that ancient organisms found at the base of the archaeobacterial branch had aerobic traits, these two enzymes are in fact part of defense mechanisms that may have evolved once oxygen had accumulated in the primitive atmosphere. In fact, evidence that anaerobiosis is an ancient trait is supported not only by the universal distribution of at least some of the glycolytic enzymes found in mainstream heterotrophic anaerobic metabolic pathways (18), but also by the identification of anaerobic eubacterial ribonucleotide reductase III as the oldest of these enzymes involved in the synthesis of deoxyribonucleotides (49). This finding suggests that the RNA to DNA evolutionary transition (39) took place in an oxygen-poor primitive environment.

The traits shared by the three main cell lines (Table 1) are far to numerous and complex to assume that they have evolved independently, i.e., they are of polyphyletic origin, or that they are the result of massive horizontal transfer. Thus, although inferences on early life may be hindered by cell fusion events or lateral flow of genetic sequences (20, 24, 59), the data summarized in Table 1 not only suggests that both in basic organization of the genetic apparatus and in its metabolic abilities eubacteria, archaeobacteria and the eukaryotic nucleocytoplasm are ultimately related and the three lines descend from a common ancestor, but also that the latter was not a protocell or any other pre-life progenitor system. It is likely that the last common ancestor of all known forms of life was in fact comparable to modern bacteria in its biological complexity, ecological adaptability, and evolutionary potential. Accordingly, the original definition of progenote cannot be used for the genetic entity from which the eubacterial, archaeobacterial, and eukaryotic branches diverged. Progenotes, if they ever existed, must have become extinct long before the separation of the three lineages, whose last common ancestor may be more appropiately described by using the term *cenancestor*, a neologism coined by Fitch and Upper (16) using a Greek prefix that can be translated both as *last* and as *common*. As discussed below, partial understanding of the processes that took place before the appearance of cenancestor may be achieved by analysing the sequences of genes that duplicated before the separation of the three major branches.

A hot origin of life?

The results summarized in the previous section indicate that the most basic questions pertaining to the origin of life relate to much simpler entities predating by a long series of evolutionary changes the cenancestor, i.e., the earliest ancestor that we can detect using rRNA-based phylogenetic trees. As noted

above, the identification of ancestral conditions is not possible for unrooted rRNA cladograms because there is no known organism that can be used as an outgroup. However, such problem can be overcomed by making outgroup comparisons using paralogous genes, i.e., genes that diverged after a duplication event and not through speciation (15), by using one set of paralogous genes as an outgroup for the other set (55).

This technique was employed a few years ago by Iwabe et al. (27) and by Gogarten et al. (21), who identified the sets that code for (i) the elongation factors (EF–G, EF–Tu) that assist in protein biosynthesis; and for (ii) the α and β hydrophilic components of F-type ATP synthethases as paralogous duplicates. Using different computing techniques, both groups independently placed the root of the universal tree between the eubacteria, on the one side, and the archaeobacteria and eukaryotes on the other. This result not only implies that the eubacteria are the oldest recognizable cellular phenotype, but also that a significant portion of the eukaryotic nucleocytoplasm is derived from archaeobacteria.

Although the eubacterial rooting of universal phylogenetic trees has been disputed and the problem remains open as one of the most challenging issues in evolutionary biology (6, 13, 17), the hypothesis that eukaryotes and archaeobacteria are sister groups is in fact supported by a number of molecular traits that are shared by their transcription machineries (30). Placing the root of universal trees at the eubacterial lineage has also groups all the known hyperthermophiles at the base of the two prokaryotic branches of rRNA trees (1, 60). This confirms previous suggestions that mesophilic eubacteria and archaeobacteria are the descendants of ancient hyperthermophiles, i.e., of organisms that grow optimally at temperatures in the range 75–100°C.

Perhaps not surprisingly, the phylogenetic distribution of heat-loving bacteria has been interpreted to support those advocating a hot origin of life. Such ideas are not new: they were discussed with considerable detail in the late 19th century by the German chemist E. Pflüger as part of his HCN-based theory on the origin of life (46). More recently, the recognition that some cyanobacteria have heat-tolerant modes of life led to Harvey (23), Copeland (10) and Scher (52) to defend the possibility of a heterotrophic emergence of life in high-temperature environments. Modern equivalents of these ideas suggest that life appeared in geothermically heated, high-temperature environments such as those found today in deep sea vents (26), or in other sites in which mineral surfaces have been hypothesized to have played a major role in the appearance of primordial chemolithoautotrophic biological systems (11, 29, 66).

However, several objections can be raised against this possibility. First of all, a high temperature regime for the origin of life would rapidly lead to an irreversible destruction of organic compounds, and thus to a very short lifetime for amino acids, purines, pyrimidines, and other biochemical compounds that are generally assumed to have been essential for the first organisms (42). It is also difficult to reconcile the possibility of an RNA world with the hot-origin-of life hypothesis (7, 35). Although the significance of ribozymes in the emergence of the biosphere is still an open question, the presence of the 2'–OH group in ribose makes RNA an extremely thermolabile polymer that is much more sensitive to cleavage than DNA at the temperatures typical for hyperthermophiles (40).

The claim that the phylogenetic distribution of hyperthermophiles supports the hypothesis that life emerged in a hot, sizzling environment (29) is in fact based on the unwarranted assumption that the root of universal evolutionary trees based on macromolecules can be extended back in time down to prebiotic

epochs. However, this may be a premature conclusion; although it is true that from a cladistic viewpoint a characteristic state found only in the deepest branches can be interpreted as primitive (61), no species exists today with all traits in the ancestral state. Indeed, heat-loving bacteria share with all other known organisms the same basic features of genome replication, gene expression, ATP-based energy producing mechanisms, and basic biosynthetic pathways. Thus, hyperthermophiles are cladistically ancient organisms, not primitive ones. This conclusion implies, of course, that a heat-loving lifestyle may be a relic of early Archean times, i.e., a secondary adaptation that evolved in population of even older mesophilic bacteria (9, 34), perhaps as a result of high temperature regimes that may have resulted from major asteroidal impacts during the late bombardment period that characterized the final stages of accretion of our planet (57) or by other, still uncharacterized selection pressure.

The confirmation of the hypothesis that hyperthermophily is indeed an ancient secondary adaptation requires an understanding of the nature of the biochemical adaptations involved in the adaptation to extreme temperatures. Although this is still a largely open question, it appears to depend on a wide spectrum of different mechanisms, which may include histone-like proteins, numerous post-transcriptional RNA modifications, high intracellular salt concentrations, multienzyme complexes protecting small intermediary metabolites, DNA-binding proteins, polyamines, and reverse gyrase, a type I topoisomerase that twists DNA into a positive supercoiled double-stranded chain (2, 9, 48, 56). Molecular cladistic analysis and a detailed understanding of the role that these and other traits play in a heat-tolerant lifestyle are required before the significance of the basal position of hyperthermophiles in phylogenetic trees is fully assessed. Until such analysis are accomplished, it is premature to take the possibility of a hot origin of life for granted..

Biological evolution before the cenancestor

Despite minor differences, the universal distribution of molecular biology processes not only provide direct evidence of the monophyletic origin of all known forms of life; they also imply that the sets of genes encoding the different components of these complex traits became frozen long time ago, i.e., major changes in them are lethal and very strongly selected against. However, it is clear that these complex, multigenic traits must have evolved through a series of simpler states. Unfortunately, no evolutionary intermediate stages or ancient simplified versions of ATP production, DNA replication and ribosome-mediated protein synthesis have been discovered in extant organisms. The absence of any known cladistically primitive taxa that originated before the freezing of these biological processes is indeed a major obstacle in the reconstruction of the sequence of the evolutionary development of Archean cells.

However, there is a way of inferring some of the characteristics of the primitive entities from which the cenancestor evolved (36). As noted above, the presence of paralogous genes implies that the last common ancestor of the three cell lineages was already endowed with two homologous genes coding for two elongation factors, as well as with F-type ATPases having homologous α and β subunits (21, 27). Accordingly, if the cenancestor had two sets of duplicate homologous genes coding for elongation factors and for the ATP-synthethase units, then it must have been preceded by a simpler cell with a

smaller genome in which only one copy of each of these genes existed, i.e., by cells in which protein synthesis required the presence of only one elongation factor, and with ATPases that lacked the α regulatory subunit (36).

Although the list of such precenancestral paralogous genes is still small (Table 1), it includes, in addition to the elongation factors and the ATPase subunits, hexameric glutamate dehydrogenases (6), glutamine synthethases (31, 64), the heat-shock protein family (22), and DNA topoisomerases I and II and DNA polymerase families A and B (17), as well as the large subunit of carbamoyl-phosphate synthase (CPSase), a protein formed by two homologous halves that resulted from an internal (i.e., partial) duplication followed by a gene fusion event (53; Lazcano, Puente, and Gogarten, in prep.). Additional products of gene pairs that may have duplicated during early Archean times have been summarized elsewhere, and include those coding for superoxide dismutases, carbamoyl transferases, dehydrogenases, pyruvate oxidase and acetohydroxyacid synthase, and several aminoacyl-tRNA synthethases (19).

In other words, detection and analysis of paralogous sequences common to the three lines are a potential source of evolutionary information which may provide direct insights to the organization and encoding capacities of genetic systems predating the cenancestor. Such paralogous sequences whose duplication preceded the cenancestor imply that before these gene amplification events, simpler living systems existed that lacked at least some of the complex regulated biochemical processes found in extant cells. That is, the cenancestor was the descendant of earlier life forms in a genetic code, and ribosome-mediated protein biosynthesis already existed, but in which (i) the large subunit of CPSase had half the molecular weight of its modern equivalent; (ii) protein biosynthesis could take place with only one elongation factor; (iii) F-type ATPases lacked the α regulatory subunit, and (iv) the DNA replication and repair machineries involved one only DNA polymerase ancestral to DNA polymerase I and II.

Precenancestral cells must have been less complex than even the simplest extant life forms, lacking the large set of enzymes and some of the sophisticated regulatory abilities of contemporary prokaryotes. Contemporary equivalents are not known, perhaps because they have been completely obliterated by their more successful descendants. However, the available databases provide direct evidence of Archean cells in which biological functions were apparently dependant on primitive, less-complex enzymes. This conclusion may be interpreted as supporting the hypothesis that early biosynthetic pathways were mediated by small, inefficient enzymes of broad substrate specificity (14, 21, 28).

Gene duplication and early cellular evolution

How was the extant set of genetic sequences built from an earlier, simpler genome? Evolution of early Archean microbes must have required important increases of their genome sizes, i.e., a major expansion of their coding abilities. The different mechanisms that may modify cellular DNA content in contemporary organisms are shown in Fig. 1. Their relative significance in prokaryotic genome size evolution should be analysed. For instance, although endosymbiosis has been a major driving force in the emergence and evolution of eukaryotic cells (41), it is unlikely that it ever played a major role in shaping prokaryotic evolution.

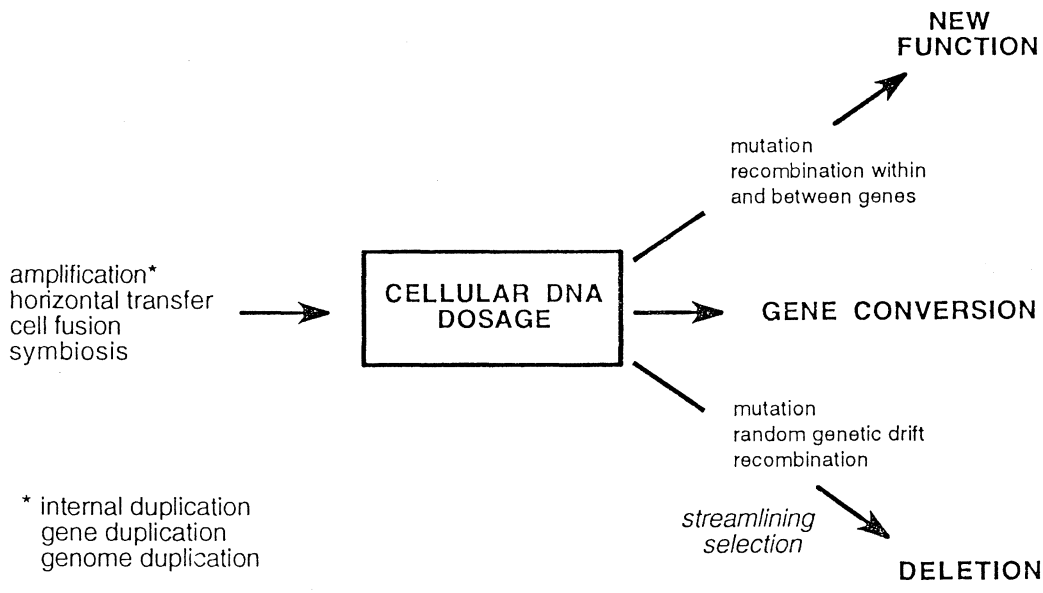

FIG. 1. Mechanisms that increase cellular DNA content and their possible evolutionary fate (43).

Moreover, cell fusion events are well-documented in *Oxytrichia* and other single-cell protists, but have not been described in bacteria. Nevertheless, it has been suggested that they may have taken place during early Archean times, thus providing with an explanation of the discrepancies between the 16S rRNA phylogeny and the protein-based trees (20, 59, 72). Besides the observation that multichromosomal mutant *Escherichia coli* cells can be obtained by blocking different stages during cell division (5), could be interpreted to support the possibility of dramatic increases in DNA content resulting from a series of bacterial genome doublings (50).

The role of horizontal transfer of genes in the expansion of the coding abilities of Archean cells should not be overlooked. The nitrogen-fixing eubacteria *Azotobacter vinelandii* is endowed with multiple copies of rather large plasmids that have increased its DNA content by a factor of 40 as compared to *Escherichia coli* (25). Evidence of lateral acquisition of genes can be recognized among different prokaryotes (58), and some of these events may have taken place shortly after the diversification of the three cell lineages (24).

However, there are several independent indications that gene duplications played a decisive role in the evolutionary development of the encoding capabilities of ancient genomes. This possibility is supported not only by the products of duplications detectable in the three cell lineages (Table 1), but also by the statistical analysis of the *Escherichia coli* sequence databases, that has shown that about 40% of the proteins whose sequence is now available are the result of duplication events (33).

If duplication was one of the major forces that shaped shaping ancient genomes, then it may be possible to understand the rapid development of levels of biochemical complexity and ecological diversity suggested by morphological and isotopic evidence showing that stromatolite-building phototactic

bacteria already existed 3.5×10^9 years ago (54). The age and complexity of these early remnants of Archean life not only suggests that the basic features of DNA-based cellular genomes had been established long before the deposition of these early fossils, but also that the major features separating the archaeobacterial and eubacterial branches were already established during the early Archean times.

Although there are many uncertainties surrounding the origin of life and the early evolution of the biosphere, the possibility that duplication events were the most important mechanism for increasing the size and complexity of prokaryotic genomes allows an estimate of the time required for the emergence of an oscillatorian-like cyanobacteria similar to the morphotypes discovered in the Warrawoona assemblage (37). Duplication events appear to occur spontaneously at relatively high, constant rates of 10^{-5} to 10^{-3} gene duplications per gene per cell generation both among eubacteria and eukaryotes (3, 51, 65). By using the lowest value of 10^{-5}, and by assuming that only 10% of the duplications are neutral, a rate of duplicon accretion of one nucleotide pair per year has been estimated, which implies that only seven million would be required to go from a 100 gene DNA/protein organism to a 7000 genes filamentous cyanobacteria (37). Of course, many of these figures are ridden by a large number of uncertainties, but they suggest that there is no compelling reason to assume that the entire process required more than 10 million years or so (37, 43). It is likely that the assumption that the emergence of life was an extremely slow process is nothing more than a deeply-rooted intellectual prejudice whose origin may be found in some of the most conservative traditions of neoDarwinism. As a matter of fact, the understanding of the mechanisms that may help to understand why the origin and evolution of early life took place in a relatively short span of geological time should be combined with the analysis of the processes underlying the lenghty periods of evolutionary stasis during which the emergence of metabolic novelties in different prokaryotic lineages has been limited or inhibited.

Concluding remarks

Because of its very nature, molecular cladistics separates clusters of adaptive characters into a nested hierarchical set which is generally expected to reflect the temporal sequence of their evolutionary acquisition. It is not surprising that such approach, which has all the demerits of a reductionist one-trait approach to biological evolution, has also led to incomplete description of cellular evolution. This limitation may be particularly clear in the failure molecular trees to include branch fusion events (i.e., anastomosis of lineages) that can describe eukaryotes as highly integrated components of evolutionary consortia, but also in the unjustified attempts to extrapolate the root of molecular phylogenies into the origin of life itself or even before (4).

However, the evidence reviewed in this paper suggests that although the analysis of macromolecular sequences cannot be extended back into prebiotic times, it is a powerful tool whose full potential may have not been fully realized. In particular, the identification of paralogous genes that duplicated before the divergence of the cenancestor can provide major insights into the nature of biological processes whose characteristics cannot be infered from the palaeontological record or from the other traditional approaches. Recognition of this possibility implies that what have been calling the root of universal trees corresponds in fact to the tip of their trunks, and in order to obtain insights into primitive cells, we must learn to read the valuable evolutionary information still contained in them.

The evidence that relevant information concerning biochemical characteristics of cells older than the three domains may be derived from ancestral paralogous genes is persuasive, and major attention should be devoted to its retrieval and interpretation. Accordingly, the design of a research strategy for the identification, sequencing and evolutionary comparison of sets of paralogous genes that originated before the separation of eubacteria, archaeobacteria and eukaryotes should be considered a major priority in our efforts to understand the evolutionary history of ancient cells, and could help to reduce in part the gap that exists in current descriptions of the evolutionary transition between the RNA world, the progenote stages, and the last common ancestor of all extant organisms.

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References

1. Achenbach-Richter, L., Gupta, R., Kandler, K. O., Woese, C. R. (1987). Were the original eubacteria thermophiles? System. Appl. Microbiol. **9**, 34–39.
2. Adams, M. W. W. (1993). Enzymes and proteins from organisms that grow near and above 100°C. Annu. Rev. Microbiol. **47**, 627–658.
3. Anderson, R. P., Roth, J. R. (1977). Tandem genetic duplications in phage and bacteria. Annu. Rev. Microbiol. **31**, 473–505.
4. Becerra-Bracho, C., Silva, E., Velasco, A. M., Lazcano, A. (1995). Molecular biology and the reconstruction of microbial phylogenies: des liaisons dangereuses? *In* Collado, J., Smith, T., Magasanik, B. (ed.), Integrative Approaches to Molecular Biology. MIT Press, Cambridge, MA, in press.
5. Begg, K. J., Donachie, W. D. (1991). Experiments on chromosome separation and positioning in *Escherichia coli*. New Biol. **3**, 1–11.
6. Benachenhou-Lahfa, N., Forterre, P., Labedan, B. (1993). Evolution of glutamate dehydrogenase genes: evidence for two paralogous protein families and unusual branching patterns of the archaebacteria in the universal tree of life. J. Mol. Evol. **36**, 335–346.
7. Benner, S. A., Cohen, M. A., Gonnet, G. H., Berkowitz, D. B., Johnsson, K. P. (1993). Reading the palimpsest: contemporary biochemical data and the RNA world. *In* Gasteland, R. F., Atkins, J. F. (ed.), The RNA World, pp. 27–70. Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY.
8. Castresana, J., Lübben, M., Saraste, M., Higgins, D. G. (1994). Evolution of cytochrome oxidase, and enzyme older than atmospheric oxygen. EMBO J. **13**, 2516–2525.
9. Confalonieri, F., Elie, C., Nadal, M., Bouthier de la Tour, C., Forterre, P., Duguet, M. (1993). Reverse gyrase: a helicase-like domain and a type I topoisomerase in the same polypeptide. Proc. Natl. Acad. Sci. USA **90**, 4753–4758.
10. Copeland, J. J. (1936). Thermophilic microorganisms. Ann. New York Acad. Sci. **69**, 328–335.
11. Danchin, A. (1990). Homeotopic transformation and the origin of translation. Prog. Biophys. Molec. Biol. **54**, 81–86.

12. Dayhoff, M. O. (1969). Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Silver Spring, MD.
13. Doolittle, W. F., Brown, J. R. (1994) Tempo, mode, the progenote and the universal root. Proc. Natl. Acad. Sci. USA **91**, 6721–6728.
14. Fani, R., Liò, P., Lazcano, A. (1995). Molecular evolution of the histidine biosynthetic pathway. J. Mol. Evol., in press.
15. Fitch, W. M. (1970). Distinguishing homologous from analogous proteins. Syst. Zool. **9**, 117–133.
16. Fitch, W. M., Upper, K. (1987). The phylogeny of tRNA sequences provides evidence of ambiguity reduction in the origin of the genetic code. Cold Spring Harbor Symp. Quant. Biol. **52**, 759–767.
17. Forterre, P., Benachenhou-Lahfa, N., Confalonieri, F., Duguet, M., Elie, C., Labedan, B. (1993). The nature of the last universal ancestor and the root of the tree of life, still open questions. BioSystems **28**, 15–32.
18. Fothergill-Gilmore, L. A., Michels, P. A. M. (1993). Evolution of glycolysis. Prog. Biophys. Molec. Biol. **59**, 105–235.
19. García-Meza, V., González-Rodríguez, A., Lazcano, A. (1994). Ancient paralogous duplications and the search for Archean cells. *In* Fleischaker, G. R., Colonna, S., Luisi, P. L. (ed.), Self-Reproduction of Supramolecular Structures: from Synthetic Structures to Models of Minimal Living Systems, pp. 231–246. Klüwer Academic Press, Dordrecht, Netherlands.
20. Gogarten, J. P. (1994). Which is the most conserved group of proteins? Homology-orthology, paralogy, xenology, and the fusion of independent lineages. J. Mol. Evol. **39**, 541–543.
21. Gogarten, J. P., Kibak, H., Dittrich, P., Taiz, L., Bowman, E. J., Bowman, B. J., Manolson, M. F., Poole, J., Date, T., Oshima, T., Konishi, L., Denda, K., Yoshida, M. (1989). Evolution of the vacuolar H^+-ATPase: implications for the origin of eukaryotes. Proc. Natl. Acad. Sci. USA **86**, 6661–6665.
22. Gupta, R. S., Singh, B. (1992). Cloning of the HSP70 gene from *Halobacterium marismortui*: relatedness of archaebacterial HSP70 to its eubacterial homologs and a model of the evolution of the HSP70 gene. J. Bacteriol. **174**, 4594–4605.
23. Harvey, R. B. (1924). Enzymes of thermal algae. Science **LX**, 481–482.
24. Hilario, E., Gogarten, J. P. (1993). Horizontal transfer of ATPase genes—the tree of life becomes a net of life. BioSystems **31**, 111–119.
25. Holloway, B. W. (1993). Genetics for all bacteria. Annu. Rev. Microbiol. **47**, 659–683.
26. Holm, N. G. (ed.) (1994). Marine Hydrothermal Systems and the Origin of Life. Klüwer Academic Press, Dordrecht, Netherlands.
27. Iwabe, N., Kuma, K., Hasegawa, M., Osawa, S., Miyata, T. (1989). Evolutionary relationship of archaebacteria, eubacteria, and eukaryotes infered from phylogenetic trees of duplicated genes. Proc. Natl. Acad. Sci. USA **86**, 9355–9359.
28. Jensen, R. A. (1976). Enzyme recruitment in evolution of new function. Annu. Rev. Microbiol. **30**, 409–427.
29. Kandler, O. (1994). The early diversification of life. *In* Bengtson, S. (ed.), Early Life on Earth: Nobel Symposium No. 84, pp. 111–118. Columbia University Press, New York, NY.
30. Klenk, H.-P., Doolittle, W. F. (1994). Archaea and eukaryotes versus bacteria. Curr. Biol. **4**, 920–922.
31. Kumada, K., Benson, D. R., Hillemann, D., Hosted, T. J., Rochford, D. A., Thompson, C. J., Wohlleben, W., Tateno, T. (1993). Evolution of the glutamine synthase gene, one of the oldest existing and functioning genes. Proc. Natl. Acad. Sci. USA **90**, 3009–3013.
32. Kluyver, A. J., van Niel, C. B. (1936). Prospects for a natural system of classification of bacteria. Zbl. Bakt. (2. Abt.) **94**, 369–393.
33. Labedan, B., Riley, M. (1994). Widespread sequence similarities among *Escherichia coli* proteins. J. Bacteriol. **177**, 1585–1588.
34. Lazcano, A. (1993). Biogenesis: some like it very hot. Science **260**, 1154–1155.
35. Lazcano, A. (1994). The RNA world, its predecessors and descendants. *In* Bengtson, S. (ed.), Early Life on Earth: Nobel Symposium No. 84, pp. 70–80. Columbia University Press, New York, NY.

36. Lazcano, A. (1994). The transition from non-living to living. *In* Bengtson, S. (ed.), Early Life on Earth: Nobel Symposium No. 84, pp. 60–69. Columbia University Press, New York, NY.
37. Lazcano, A., Miller, S. L. (1994). How long did it take for life to begin and evolve to cyanobacteria? J. Mol. Evol. **39**, 546–554.
38. Lazcano, A., Fox, G. E., Oró, J. (1992). Life before DNA: the origin and evolution of early Archean cells. *In* Mortlock, R. P. (ed.), The Evolution of Metabolic Function, pp. 237–295. CRC Press, Boca Raton, FL.
39. Lazcano, A., Guerrero, R., Margulis, L., Oró, J. (1988). The evolutionary transition from RNA to DNA in early cells. J. Mol. Evol. **27**, 283–290.
40. Marguet, E., Forterre, P. (1994). DNA stability at temperatures typical for hyperthermophiles. Nucleic Acid Res. **22**, 1681–1686.
41. Margulis, L. (1993). Symbiosis in Cell Evolution (2nd. ed.). W. H. Freeman and Co., New York, NY.
42. Miller, S. L., Bada, J. L. (1989). Submarine hot springs and the origin of life. Nature **334**, 609–611.
43. Miller, S. L., Lazcano, A. (1994). From the primitive soup to cyanobacteria: it may have taken less than 10 million years. *In* Doyle, L. (ed.), Circumstellar Habitable Zones. California University Press, Berkeley, CA, in press.
44. Nuttall, G. H. F. (1904). Blood Immunity and Blood Relationship: a Demostration of Certain Blood-Relationships amongst Animals by Means of the Precipitin Test for Blood. Cambridge University Press, Cambridge, United Kingdom.
45. Oparin, A. I. (1938).The Origin of Life. MacMillan Co., New York, NY.
46. Oró, J., Lazcano-Araujo, A. (1981). The role of HCN and its derivatives in prebiotic evolution. *In* Vennesland, B., Conn, E. E., Knowles, C. J., Westley, J., Wissing, F. (ed.), Cyanide in Biology, pp. 517–541. Academic Press, New York, NY.
47. Razin, S. (1978). The mycoplasmas. Microbiol. Rev. **42**, 414–470.
48. Reeve, J. N. (1994). Thermophiles in New Zeland. ASM News **60**, 541–545.
49. Reichard, P. (1993). From RNA to DNA, why so many ribonucleotide reductases? Science **260**, 1773–1777.
50. Riley, M., Anilionis, A. (1980). Evolution of the bacterial genome. Annu. Rev. Microbiol. **32**, 519–560.
51. Schimke, R. T., Sherwood, T. W., Hill, A. B. (1986). The rapid generation of genomic change as a result of over-replication. Chemica Scripta **26B**, 305–307.
52. Scher, S. (1959). Thermal factors in archaeometabolism. *In* Oparin, A. I., Pasynskii, A. G., Braunshtein, A. E., Pavlovskaya, T. E. (ed.), The Origin of Life on the Earth, pp. 650–651. Pergamon Press/ MacMillan Co., New York, NY.
53. Schofield, J. P. (1993). Molecular studies on an ancient gene encoding for carbamoyl-phosphate synthethase. Clin. Sci. **84,** 119–128.
54. Schopf, J. W. (1993). Microfossils of the early Archean apex chert: new evidence of the antiquity of life. Science **260**, 640–646.
55. Schwartz, R. M., Dayhoff, M. O. (1978). Origins of prokaryotes, eukaryotes, mitochondria, and chloroplasts. Science **199**, 1395–1403.
56. Segerer, A. H., Burograf, S., Fiala, G., Huber, G., Huber, R., Pley, U., Stetter, K. O. (1993). Life in hot springs and hydrothermal vents. Orig. Life Evol. Bios. **23**, 77–90.
57. Sleep, N. H., Zahne, K. J., Kastings, J. F., Morowitz, H. J. (1989). Annihilation of ecosystems by large asteorid impacts on the early Earth. Nature **342**, 139–142.
58. Smith, M. W., Feng, D. F., Doolittle, R. F. (1992). Evolution by acquisition: the case for horizontal gene transfers. Trends Biochem. Sci. **17**, 489–493.
59. Sogin, M. L. (1991). Early evolution and the origin of eukaryotes. Curr. Opin. Gen. Develop. **1**, 457–463.
60. Stetter, K. O. (1994). The lesson of archaebacteria. *In* Bengtson, S. (ed.), Early Life on Earth: Nobel Symposium No. 84, pp. 143–151. Columbia University Press, New York, NY.
61. Stevens, P. F. (1980). Evolutionary polarity of character states. Annu. Rev. Ecol. System. **11**, 333–358.
62. Takao, M., Kobayashi, T., Oikawa, A., Yasui, A. (1989). Tandem arrangements of photolyase and superoxide dismutase genes in *Halobacterium halobium*. J. Bacteriol. **171**, 6323–6329.

63. Takao, M., Oikawa, A., Yasui, A. (1990). Characterization of a superoxide dismutase gene from the archaebacterium *Methanobacterium hermoautotrophicum*. Arch. Biochem. Biophys. **283**, 219–216.
64. Tiboni, O., Cammarano, P., Sanangelantoni, M. A. (1993). Cloning and sequencing of the gene encoding glutamine synthase I from the archaeum *Pyrococcus woesei*: anomalous phylogenies infered from analysis of archeal and bacterial glutamine synthase I sequences. J. Bacteriol. **175**, 2961–2969.
65. Tlsty, T. D., Albertini, A. M., Miller, J. H. (1984). Gene amplification in the *lac* region of *E. coli*. Cell **37**, 217–224.
66. Wächsterhäuser, G. (1990). The case for the chemoautotrophic origins of life in an iron-sulfur world. Orig. Life Evol. Bios. **20**, 173–182.
67. Wallace, D. C., Morowitz, N. H. (1973). Genome size and evolution. Chromosoma **40**, 121–126.
68. Woese, C. (1983). The primary lines of descent and the universal ancestor. *In* Bendall, D. S. (ed.), Evolution from Molecules to Man, pp. 209–233. Cambridge University Press, Cambridge, United Kingdom.
69. Woese, C. R. (1987). Bacterial evolution. Microbiol. Rev. **51**, 221–271.
70. Woese, C. R., Fox, G. E. (1977). The concept of cellular evolution. J. Mol. Evol. **10**, 1–6.
71. Ycas, M. (1974). On the earlier states of the biochemical system. J. Theor. Biol. **44**, 145–160.
72. Ziilig, W., Palm, P., Klenk, H. P. (1992). A model of the early evolution of organisms: the arisal of the three domains of life from the common ancestor. *In* Hartman, H., Matsuno, K. (ed.), The Origin and Evolution of the Cell, pp. 163–182. World Scientific Co., Singapore.
73. Zuckerkandl, E., Pauling, L. (1965). Molecules as documents of evolutionary history. J. Theoret. Biol. **8**, 357–366.

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From spontaneous generation to auto-organization. One hundred years of the death of Pasteur

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Summary

The problem of spontaneous generation occupied the attention over the centuries of many scientists and philosophers. This question has remained insoluble for a long time even by experimental means. It appears that the work of Pasteur undoubtedly led to major advances in the field of microbiology, but not in the field of the origin of life; spontaneous generation is an issue which stems from the work of Oparin, not Pasteur. The theory of self-organization is related since antiquity with spontaneous generation so that today we can see that the "artificial life" originated by the work of von Neuman appears to be a modern kind of spontaneous generation controversy.

Key words: auto-organization, spontaneous generation, protoplasm, Pasteur, von Neuman machine

Resumen

El problema de la generación espontánea ha ocupado la atención de muchos científicos y filósofos durante siglos. Este problema fue irresoluble durante mucho tiempo, incluso por métodos experimentales. El trabajo de Pasteur sobre el tema fue, indudablemente, un gran avance en el campo de la microbiología, pero no en el campo del origen de la vida: el conocimiento sobre la generación espontánea de la vida surge de las ideas de Oparin, no de las de Pasteur. La teoría de la auto-organización se ha relacionado desde la antigüedad con la idea de la generación espontánea, e incluso podemos ver hoy día que el tema de la "vida artificial", originado por el trabajo de von Neuman, ha pasado a ser la versión moderna de la controversia sobre la generación espontánea.

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Introduction

The notion of spontaneous generation has varied with the times, schools of scientific thought, and philosophical and theological concepts. A uniform vision is always tacitly presented to us. Spontaneous generation is the sudden appearance of life from specific entities, independent of any parent. Nevertheless, important differences exist relative to the nature of these entities. When the starting entities are inorganic matter, one refers to "abiogenesis"; when they are organic, deriving from living matter, one refers to "heterogenesis". Ever since Aristotle, and up to the 19th century, heterogenesis was the main concern. After 1859, with the publication by Darwin of «The Origin of Species», German and English biologists expanded this debate in more modern terms and in connection with the theory of evolution. The debate no longer solely concerned problems of generation, but through abiogenesis it also encompassed the origin of life as a scientific problem.

In France, even though the notion of spontaneous generation was shaken several times by the works of Redi, Spallanzani and Schwann, history has preferentially given credit to the work of Pasteur for demonstrating that living organisms cannot appear spontaneously from inanimate matter (15).

The first observations

The originality and the wealth of numerous texts dating from the Greco-Latin period are well known: Anaximandre of Milet (610 to 547 B.C.) was the first to assert that the Earth is round, but he favored the idea that all creatures on Earth emanated from mud heated by the Sun. "When the Earth first became warm, in the abysses where hot and cold come together, numerous living creatures appeared [...] extracting their matter from slime".

The theory of spontaneous generation developed by Aristotle (384 to 322 B.C.) long remained the sole reference of the occidental world. Indeed, it was the only theory compatible with one of the central dogmas of Christianity, in particular of the Catholic church, most likely because only a small step needed to be taken to go from "spontaneous generation" to the immaculate conception!

According to the theory of spontaneous generation, living creatures are born spontaneously from inanimate matter. According to an ancient Egyptian belief, crocodiles are born from the hot mud of the Nile, whereas the first animals originate from marine slime dried by the sun.

From Antiquity up to the Middle Ages and the Renaissance, living creatures were believed to originate by spontaneous generation from mud, decaying matter, etc. The physician and alchemist, Jean-Baptiste van Helmont (1580–1644), a contemporary of Descartes, is the author of a famous recipe for the production of mice: "[...] if one squeezes out a dirty shirt [...] where there is wheat matter, in about twenty days the matter extracted from the shirt becomes altered by the smell of the grain, transmutates the wheat surrounded by its husk into a mouse [...]" (28). In 1668, it was the Italian biologist and poet Francesco Redi (1626–1697), who, without directly rejecting spontaneous generation, finally demonstrated by a series of experiments that the larvae of flies do not spontaneously appear on meat. After having placed pieces of meat in a jar, he observed that when the jar was covered by gauze no larvae

develop, whereas when it was left uncovered, maggots proliferated. He concluded that the maggots simply arose from eggs deposited by the flies. Another Italian scientist, Antoine Vallisneri, confirmed this observation by performing the same experiment with larvae from fruit flies. The experiment of Redi was one of the first in the period, spanning two centuries (up to the time of Pasteur), which was determinant in discarding the idea of spontaneous generation.

At the same time, thanks to the perfection of an ingenious tool, the microscope, experimentation and the observation of nature developed. In spite of the lack of sharpness of the images observed, this invention spread rapidly, and largely contributed to progress in scientific thinking and in re-instating facts derived from observation.

As he was observing through his microscope a thin slice of cork placed on a black background and strongly lighted from above, the versatile English scientist Robert Hooke (1635–1703), noticed that it was riddled with minute cavities, the "cells", as he called them.

Using instruments that he had made himself, the Dutch craftsman, Antoine van Leeuwenhoek (1632–1723), discovered and observed blood cells, nerve and muscular tissues, and then in collaboration with Louis de Hamm, spermatozoa which he considered microscopic worms or small animals.

It is indeed thanks to the microscope that the problem of "generation" was truly approached. The generation of living organisms was one of the major thrusts of research in the 17th and 18th centuries. This is seen in the many reports by Van Leeuwenhoek concerning spermatozoa, these "animalcules" in which "one can see" the future animal (the theory of preformation and of spontaneous generation).

In the second half of the 18th century, experiments were again undertaken; spontaneous generation no longer concerned mice, but microscopic organisms. Very soon, a fierce controversy arose between two priests, John Turbeville Needham (1713–1781) and Lazzaro Spallanzani (1729–1799), who had totally diverging opinions in this matter. Needham placed decaying substances in flasks hermetically sealed with a cork stopper, and heated it. After cooling, "animalcules" swarmed. Needham, supported by Georges-Louis Leclerc de Buffon (1707–1788), considered them to arise by spontaneous generation, that is by the auto-assembly of organic molecules triggered by a vegetative force. Spallanzani repeated the same experiment in 1765, but the flasks were heated longer, and they were better closed by sealing the neck of the flasks with a flame: no animalcules appeared.

It is noteworthy that all these experiments and observations were not carried without method: on the contrary, they clearly evoke the experiments that Pasteur performed a century later.

Since the position of Buffon and Needham did not involve the intervention of God, it was considered by their contemporaries as materialistic and atheist. The link between belief in spontaneous generation and atheist and materialistic philosophy was upheld throughout the century. As we shall see later, in 1863 Félix Archimède Pouchet, an opponent of Pasteur in this field, was blamed of wanting to shatter the foundations of religion by advancing arguments in favor of pantheism and materialism.

At the end of the 18th century and in the beginning of the 19th century, Jean-Baptiste de Lamarck (1744–1829) asserted the historical dimension of nature (10, 11). The transformism of Lamarck is above all the production of living matter by the laws of physics only, beginning with the simplest organisms from which the more complex ones are formed, up to the present living organisms. Lamarck, a friend of Buffon, believed that only the simplest living creatures (which he named "infusoria") appear by spontaneous generation (which he preferred to call "direct generation"), and that all other forms derive from them. He even proposed that direct generation results from the organization by electricity or heat

of a "small gelatinous mass". This position was in perfect agreement with the naturalist ideas of the author for whom life does not require divine intervention, since the laws of nature are sufficient. This in fact is the main justification of his theory of transformism: the most complex forms must derive from the first and simplest forms by an extremely long process.

Schwann, Pasteur and the advent of the experimental method

The German scientist Theodor Schwann (1810–1882), strongly rejected vitalism and the theory of spontaneous generation. Having become famous through his studies on the theory of the cell, in which he proposed that all living organisms are made up of cells containing a nucleus, his ideas were rapidly adopted by others. He became convinced that "the forces acting on living matter are the same as on inorganic matter".

This is how Schwann performed his first experiments on spontaneous generation, in 1836. The decisive role played by oxygen of the air in the production of germs was established at that time. Indeed, it was known that one can interrupt the development of microorganisms by intense heating of the culture medium, and that growth will begin again as soon as oxygen or air is introduced into this medium. Schwann demonstrated that pre-heating the oxygen or air prevents the development of microorganisms. This demonstration did not prevent Pouchet (26) from declaring that under the same conditions, he had obtained exactly opposite results!

This was the beginning of a major controversy, a prelude to the work of Pasteur (23).

In August 1857, Louis Pasteur (1822–1895) undertook a series of experiments to test the results obtained by Schwann. On January 30, 1860, the Academy of Sciences decided to reward by a prize, the "Prix Alhumbert", for the experiments that had the most demonstrably brought new light on the question of spontaneous generation. Pasteur received this prize, and described his experiments in his Journal in 1861:

"In a series of flasks of 250 cubic centimeters, I introduced the same foul liquid [...] so that it occupied about one third of the total volume. I stretched out the necks of the flasks with a flame, I brought the liquid to a boil, and then sealed the flasks as the liquid was boiling. Vacuum was created in the flasks; I then broke off their tip in a specific place. The outside air rushed in with violence, bringing all the dust associated with it. I then immediately sealed the flasks by brief treatment with the flame, and I transported them to an incubator at 25 or 30°C, that is, in the best conditions possible for the development of animalculi and molds."

Having performed these experiments in Paris, then in the Jura mountains and on a glacier—to test the influence of the environment—Pasteur concluded in 1861 in a lecture given at the Chemical Society of Paris: "I feel confident that I have rigorously demonstrated that in all the experiments where it was believed that spontaneous generation could be observed among lower forms of life, the subject of this debate, the observer was victim of illusions or of errors that he did not notice, or was unable to avoid".

At the time of Pasteur, the conservatives and the French catholics related the theory of spontaneous generation and Darwinism to other radical views. It appears that Pasteur himself a conservative, had undertaken his experiments partly in support of traditional views; in April 1864, he stated "what a victory

it would be for materialism, if it could be demonstrated that matter can auto-organize itself and alone create life".

This situation grew in importance throughout the 19th century, and the climate was further degraded by the publication of Darwin's book (4) in French. The book appeared in 1862, and the translator was Clémence Royer, who adhered to all the doctrines the most despised by the conservatists: atheism, materialism and republicanism.

From embarrassment to confusion

The theory of evolution based on natural causes developed by Darwin in the decade of 1850 implied an origin of abiogenetic life. Whereas the materialists declared that this implication of Darwinism validated their philosophical position, most French biologists considered this to be an embarrassing situation. In a way, they were being forced to accept abiogenesis, at a time when experimental discoveries clearly demonstrated the impossibility of the existence of heterogenesis!

In fact, ever since Darwin and for a number of years thereafter, only the most progressive considered as a fact that life could have originated from an inorganic world. At the end of the 19th century, chemistry was still not sufficiently advanced to be able to consider an experimental approach of chemical evolution. Certainly, the work of Friedrich Wöhler (1800–1882) had demonstrated as early as 1828 that an organic compound, urea, could be synthesized from inorganic matter, ammonium cyanate, but these results were not linked to problems of the origin of life. These experiments constitute however a first step, the one that should have changed the course of ideas, by demonstrating that it is possible to pass from the mineral to the organic world. A few years later, in 1834, Justus von Liebig (1803–1873) by allowing potash to interact with carbon monoxide obtained oxalic acid, an organic compound widely spread among fungi and molds. Butlerow (3) and later Fischer succeeded in synthesizing sugars.

Little by little prejudices decreased, but such a change did not come about without some resistance, including from among respected scientists.

The confusion which prevailed during the 19th century is reflected by the variety of terms used to designate the passage from the lifeless to the living. Among them are: abiogenesis, used by Huxley (9); autogony, by Haeckel (7); archaeobiosis, by Bastian; etc. The most common term used by German scientists was *Urzeugung*, meaning literally primitive generation (*Ur* means primitive or primordial, and *Zeugen* means to produce or generate). During that time, the literal translation was spontaneous generation, even if it was clear within this context, that a gradual rather than a sudden transition was meant!

This analysis allows us to stress the fact that the work of Pasteur undoubtedly contributed to our understanding of how microorganisms are reproduced and transmitted for one medium to another; it led to major advances in the field of microbiology, but not in the field of the origin of life. One can even agree with R. Moreau (15) that it delayed work on the origin of life. Indeed, it was not until Oparin, Haldane, Urey, Fox, Oró, Miller,... (8, 14, 18, 19, 22) some 60 years later, that experiments were reinitiated in this area.

Protoplasmic theories

In a report which he made in 1868 to the British Association for the Advancement of Science in Edinburgh, the British scientist Thomas Henry Huxley, a great admirer of the work of Pasteur, declared that all living beings stem from a unique substance, the protoplasm, itself originating from inorganic matter. The protoplasm makes up the mass of the cell, it is distinct from the cell membrane and the nucleus, and represents the elementary substance, the physical basis of life. According to Huxley, the properties of the protoplasm "transcend" the properties of the different parts of the cell; as it is believed to be the basis of plants and animals, it cannot be subdivided without resulting in the destruction of vital metabolic functions. For Thomas Graham (1805–1869), who at the same time developed the theory of colloids, life results from the colloidal state that matter can adopt. On his side, the German scientist Ernst Haeckel (1834–1919), an enthusiastic defender of Darwin, placed the problem of the origin of life in an evolutionary context. He described a process that would occur in two phases: mineral matter would first be transformed into organic matter of increasing complexity; this matter would then auto-organize itself into a primitive "Monera", a kind of nucleus-free cell which would represent the simplest form of living organic matter. Darwin wrote to him about this process in 1868: "At times, your audacity makes me shiver".

The hypothesis of the leader of the German evolutionist movement is based on the postulate that, in epistemological terms, there is no fundamental discontinuity between the animate and the inanimate world, that all vital phenomena are governed by the same rules as inanimate phenomena. He considered that the advent of the theory of evolution earmarks a new era in the history of the human mind. It implies abandoning religious beliefs and adopting a new philosophy, monism, as opposed to dualism between the body and soul, the basic principle of Man's conception. In 1904 he described in his treaty "The Enigma of the Universe" what he considered as the major conflict of philosophy in history, namely the conflict between monism and dualism. Dualism cleaves the universe into two distinct substances, it separates the body from the soul, matter from energy; traditionally, it is linked to teleological or idealistic dogmas. Monism, on the other hand, considers only one substance in the universe, it is in line with the mechanistic theory and the idea of unity in nature.

Later, Oparin, in the historically famous chapters of his book «The Origin of Life on Earth» violently contested the raw mechanicism of Haeckel, who apparently made no distinction between the formation of a crystal and the formation of a living cell. The seeming absence of complexity in the moners of Haeckel had already been strongly criticized by his contemporaries Nägeli and Weismann (17, 29). The Swiss botanist Karl Nägeli proposed two distinct phases, the formation and the accumulation of proteinaceous matter, and its organization in a kind of colloid which he named micelle. It is starting from this mass of protoplasm that the first cellular forms, which Nägeli called probionts, would develop. August Weissman, one of the principal founders of what is known today as Darwinism, considered that to explain the origin of life, spontaneous generation of protoplasm is required "For me, I admit, spontaneous generation remains a logical postulate, in spite of the failure of all the attempts to demonstrate it".

At the end of the 19th century, and throughout the first half of the 20th century, the "living particles" that are the protoplasm had several names: "gemmules" for Darwin, "colloids" for Graham, "monera or plastiduls" for Haeckel, "micelles" for Nägeli,... However, neither Haeckel, nor Nägeli, nor anyone else

proposed a mechanism to explain how the protoplasm or its proteins are formed from inorganic matter. According to Nägeli, the synthesis of proteins cannot be detected because "it probably takes place under a thin layer of clay or sand". Eduard Pflüger (25) was the only one to suggest the cyanide radical (CN) as the possible link between the inanimate and the animate world. He stressed the fact that in the laboratory "the CN radical is obtained when an electric discharge passes through a nitric acid solution and when nitrogen is brought into contact with incandescent carbon. Such compounds must have been made when the Earth was still composed of live coals". With Pflüger, the idea arose that life would have been preceded by a long chemical evolution.

In spite of this, the subject was only discussed in qualitative terms, and the theories on the origins of life remained vague. Edmond Perrier (24) could dream about chemical syntheses of nitrogen compounds comparable to egg-white, a kind of protoplasm that would one day emerge alive from the flasks of chemists: "even if this substance emerges as an amorphous and undefined mass, could one not give it the shape and reproductive activity that are the essence of a living organism? [...]".

The development of biochemistry at the turn of this century demonstrated the chemical complexity of the living cell. In particular the fact that specific enzymes, isolated in solution, could catalyze metabolic reactions shook the theory whereby the protoplasm as a whole was responsible for vital functions. It thus became clear that the origins of life could not possibly be explained in terms of the origin of only one molecular species, regardless of how complex this species was. It became necessary to search for the origin of systems composed of several molecules acting in a coordinated manner. As the living cell progressively revealed its complexity, the mystery of the origins of life seemed more difficult to elucidate.

A modern kind of spontaneous generation: the auto-organisation controversy

Molecular biology made it possible to gain intimate insight into genetic information, and replication and expression of this information. These notions, as developed today, can be interpreted in more general terms and in the strict theory of auto-replicative systems. In the 30s, before the advent of molecular biology, the mathematician John von Neuman (1903–1957) (2) had considered in abstract terms the possibility of building a computer or an automate capable of auto-replication, just as living cells replicate themselves. He demonstrated that such an automate could be constructed if one had at one's disposal four compounds. If one assumes that DNA would supply the instructions, DNA polymerase would be the duplicator, tRNAs would translate and the ribosomes would be the assembly machine, then the present-day cells comply with the requirements of Von Neuman, even though it seemed highly improbable that such a complex system could have appeared in one step. The idea of a gradual sequence of events such that the system would be viable at every step of its evolution, would imply that any one of its functions taken separately would originally have been obtained by the same structure. For instance, it has been suggested that primitive RNA could have combined functions of stocking information and of translation (12, 20, 21), and this has been perfectly illustrated by the recent discovery of ribozymes (6), and by the experiments of Harry Noller concerning the peptidyl-synthetase activity of ribosomal RNA. Starting from here, two parallel lines of research can be envisaged:

The first one considers the origins of life in terms of spontaneous and sudden generation of a first nucleic acid. It was Hermann Muller (1890–1967) (16) who, in 1926, first suggested that life could have

arisen from a gene. The debate was now between those who proposed a protoplasmic origin of life (the so-called "organism-as-a-whole" view of life), and those for whom the origin of life is identified with the origin of genetic material which arose by a fortuitous chemical combination. For Muller, and for a large number of geneticists and molecular biologists today, the gene is the only possible "living molecule" capable of reproducing itself and of producing metabolic enzymes. Hence, the basic question is whether life appeared in one decisive step (as postulated by Muller) or through a long gradual process (as maintained by Oparin). The problem of spontaneous generation is now compacted (or reduced) to the question of time. Oparin saw the origin of life as a transition between the nonliving and living, but the spontaneous production of a functional living entity, be it a frog, a bacterium, a virus or a living molecule, did not appear suddenly from nonliving matter. Life appeared slowly by a long process.

Another approach, in line with the ideas of Von Neuman, developed itself in areas bringing together physical-chemistry, biology, and cybernetics, and is presently to be found in artificial life. The term of "organism" is often replaced by that of "auto-organizer system", and for some, such as Henry Atlan (1), auto-organization corresponds to the hypothesis of a self-programming program. For others, the concept of auto-organization signifies the capacity of a physico-chemical system that only exchanges energy with its surroundings, to organize itself in a spatio-temporal manner. For Prigogine (27), the thermodynamicist, "in open systems, dissipative and irreversible movements sometimes give rise to ordered structures [...]". For him, this phenomenon is equivalent to auto-organization of the living.

Experimentally, it is possible to synthesize the first elements of life indispensable for the production of the first program, but the first auto-replicative molecule was never obtained de novo. Scientists such as Katchalsky, Eigen (5) and Prigogine searched for and are still searching for laws of physico-chemical organization showing that the origin of life is an event that could have occurred every time the physico-chemical conditions of the primitive world were suitable. The question is to determine the laws that govern the exchanges and structure of the system so that starting from an unorganized state, there would be an automatic evolution towards more heterogeneity, diversity and complexity.

Biochemistry made it possible to prove that perfect identity exists between the nature of matter in the living and nonliving. Nevertheless, certain physico-chemical characteristics exist that are specific to living beings and link them to each other. This does not mean that one can reduce everything to a physico-chemical structure, that one can reduce the living to only a chemical machine. The specificity or the originality of the living resides no more in physico-chemical laws than in a vital force.

The first living entity represented physico-chemical continuity with the prebiotic medium; life appeared only from having acquired a certain autonomy, that is its own laws, different yet compatible with the laws of matter. For this reason, the study of the origins of life should be concerned with a biology that studies life, rather than simply the matter of the living. This problem has still not been resolved by modern biology.

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References

1. Atlan, H. (1992). L'organisation biologique et la théorie de l'information. Hermann Ed., Paris, France.
2. Burks, A. W. (1966). Theory of Self-Reproducing Automata. University of Illinois Press, Urbana, IL.
3. Butlerow, A. (1861). Formation synthétique d'une substance sucrée. Comptes Rendus Académie des Sciences **53**, 145–147.
4. Darwin, C. (1859). De l'origine des espèces au moyen de la sélection naturelle ou la préservation des races favorisées dans la lutte pour la survie. C. Reinwald et al. (ed.), English translation by E. Barbier, 1980. Paris, Maspero.
5. Eigen, M. (1971). Self-organization of matter and the evolution of biological macromolecules. Naturwisssenschaften **58**, 465–523.
6. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., Altman, S. (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell **35**, 849–857.
7. Haeckel, E. (1868). Natürliche Schöpfungs-Geschichte (Berlin). English translation by E. Lankester as The History of Creation or the Development of the Earth and its Inhabitants by the Action of Natural Causes. New York, 1876.
8. Haldane, J. B. S. (1929). The origin of life. Rationalist Annual New Biology **16**, 12–27.
9. Huxley, T. H. (1868). On the physical basis of life, in Lay sermons, addresses and reviews (New York, 1883), p. 129.
10. Lamarck, J. B. de (1815). Histoire naturelle des animaux sans vertèbres, tome I, 123–125. Verdière, Paris, France.
11. Lamarck, J. B. de (1983). Additions à la philosophie zoologique, tome II. Ed. Culture et Civilisation, Bruxelles, Belgium.
12. Lazcano, A., Guerrero, R., Margulis, L.,Oró, J. (1988). The evolutionary transition from RNA to DNA in early cells. J. Mol. Evol. **27**, 283–290.
13. Maurel, M. C. (1994). Les origines de la vie. Syros, Paris, France. (See also all references included.)
14. Miller, S. L. (1953). A production of amino acids under possible primitive Earth conditions. Science **117**, 528–529.
15. Moreau, R. (1992). Les expériences de Pasteur sur les générations spontanées. La Vie des Sciences. Comptes Rendus, vol. **9**, n. 4, p. 312.
16. Muller, H. J. (1926). The gene as the basis of life. Proceedings of the International Congress of Plant Science, Ithaca, NY.
17. Nägeli, C. W. von (1884). A mechano-physiological theory of organic evolution. Open Court Publishing Co., Chicago, IL.
18. Oparin, A. I. (1922). Communication to the Congress of the Russian Botanical Society, Moscow, Russia.
19. Oparin, A. I. (1956). The Origin of Life on the Earth. Oliver and Boyd, Edinburgh, United Kingdom.
20. Orgel, L. E. (1987). Evolution of the genetic apparatus: a review. Cold Spring Harbor Symp. Quant. Biol. **52**, 9–16.
21. Orgel, L. E. (1994). L'origine de la vie sur terre. Pour la Science **206**, 80–88.
22. Oró, J. (1965). Stages and mechanisms of prebiological organic synthesis. *In* Fox, S. W. (ed.), The Origin of Prebiological Systems and their Molecular Matrices, pp. 137–161. Academic Press, New York, NY.
23. Pasteur, L. (1869). Nouvelles recherches sur les découvertes microscopiques et la génération des corps organisés. Oeuvres, vol. 2. Masson et Cie., Paris, France.
24. Perrier, E. (1920). La Terre avant l'histoire. La Renaissance du Livre, Paris, France.
25. Pflüger, E. (1875). Beitrage zur lehre von der respiration. Archiv. ges. physiol. **10**, 251–367.

26. Pouchet, F. A. (1859). Hétérogénéité ou traité de la génération spontanée. Baillère, Paris, France.
27. Prigogine, I., Nicolis, G. (1971). Biological order, structure and instabilities. Quart. Rev. Biophysics **4**, 107–148.
28. Van Helmont, J. B. (1671). Des principes de la physique, 104. Oeuvres de Jean Baptiste Van Helmont traitant des principes de médecine et de physique pour la guérison des maladies, traduction de Jean Le Conte, chez Jean Antoine Hugueton et Guillaume Barbier.
29. Weismann, A. (1889). Essay upon Heredity. Oxford University Press (Clarendon), London, United Kingdom.

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Peptide Nucleic Acid (PNA): a model structure for the primordial genetic material?

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Summary

We have recently described a novel DNA analog, peptide nucleic acid (PNA), which is relevant for the discussion of the origin of life. PNA consists of a pseudo-peptide (polyamide) backbone comprised of (2-aminoethyl)glycine units to which nucleobases are attached via carbonyl methylene linkers. We have found that PNA binds to oligo(deoxy)-ribonucleotides obeying the Watson-Crick base pairing rules, i.e., A–T and G–C base pairs are highly preferred. Thus in a chemical sense (but not in a functional one) PNA bridges the gap between proteins and nucleic acids. The results obtained with PNA clearly show that molecules with the potential of carrying genetic information are not required to contain either phosphates or sugars but could be "peptides". It should be emphasized that although we have shown that PNA with the (2-aminoethyl)glycine backbone is an amazingly good mimic of DNA, we do not yet know the structural constraints within which this property exists. Recent results have shown, however, that extending the backbone by using β-alanin in place of glycine is deleterious for PNA/DNA complex stability. It can be argued that the optimal backbone for a genetic material is not necessarily the backbone that gives the most stable duplex. Indeed, if the duplex is too stable, the genetic information is not available for transcription and replication processes. It is proposed that the primordial genetic material could have been PNAs, i.e., DNA analogues having a peptide backbone. PNA monomers based on the amino acids α,γ-diaminobutyric acid or ornithin are suggested as compounds that could have been formed in the prebiotic soup. Finally, the possibility of a PNA/RNA world is presented, in which PNA constitutes the stable genetic material, while RNA, which may be polymerized using the PNA as template, accounts for enzymatic activities, including PNA replication.

Key words: PNA, DNA analog, replication, prebiotic compounds, primordial genetic material

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Resumen

Hemos descrito recientemente un nuevo análogo del DNA, el péptido ácido nucleico (PNA), que puede tener importancia para la discusión sobre el origen de la vida. El PNA está formado por un esqueleto de pseudo-péptido (poliamida) compuesto por unidades de (2-aminoetil)glicina, al cual están conectadas las nucleobases mediante uniones metilcarbonilos. Hemos encontrado que el PNA se une a los oligo(desoxi)-ribonucleótidos obedeciendo las reglas de Watson-Crick de emparejamiento de bases, es decir, que los pares A–T y G–C muestran una especial preferencia. Por consiguiente, desde el punto de vista químico (pero no desde el funcional) el PNA rellena el hueco entre proteínas y ácidos nucleicos, y los resultados obtenidos con el PNA demuestran claramente que las moléculas capaces de transportar información genética no tienen por qué poseer fosfatos o azúcares, sino que pueden ser "péptidos". Debe destacarse que, aunque hemos demostrado que el PNA con el esqueleto de (2-aminoetil)glicina es un equivalente sorprendentemente bueno del DNA, aún no conocemos las limitaciones estructurales que puede presentar. Resultados recientes han demostrado, sin embargo, que extender el esqueleto mediante β-alanina en lugar de glicina resulta deletéreo para la estabilidad del complejo PNA/DNA. Podría responderse que el esqueleto óptimo de un material genético no es necesariamente el esqueleto que proporciona el duplexo más estable. Por supuesto, si el duplexo es demasiado estable, la información genética no está disponible para los procesos de transcripción y replicación. Se propone que el material genético primordial podrían haber sido PNAs, esto es, análogos del DNA con un esqueleto peptídico. Los monómeros de PNA basados en los aminoácidos α,γ-diaminobutírico u ornitina son compuestos que podrían haberse formado en la sopa prebiótica. Finalmente, se discute la posibilidad de que haya existido un mundo PNA/RNA, en el cual el PNA constituía el material genético estable, mientras que el RNA, que podía polimerizarse utilizando el PNA como molde, era el responsable de las actividades enzimáticas, incluyendo la replicación del PNA.

PNA characteristics

Several DNA-mimicking molecules with backbones that do not contain phosphates or sugars have been described (12), and should be considered in discussions of possible prebiotic genetic materials. PNA (peptide nucleic acid) is such a DNA analog, having an achiral, charge-neutral, pseudo-peptide backbone (Fig. 1) (7, 13). It was not made in a prebiotic context, but was designed as a reagent that sequence specifically recognize double stranded DNA. The PNA molecule has a backbone composed of glycine units, extended at the glycine amine by an aminoethyl group. The connection to the nucleobase, which is essentially an acetic acid, is positioned on the amino group of the glycine. PNA molecules are synthesized by conventional peptide chemistry using adequately protected monomers for each of the four nucleobases (Fig. 2) (4, 5).

The DNA-mimicking properties of PNAs have been studied by thermal stability measurements, the technique usually employed when studying the stabilities of nucleic acid hybrids. It was found that complexes between PNA and DNA (or RNA) oligonucleotides in the antiparallel configuration (PNAs are oriented amino to carboxy terminal) are approximately one degree Celsius more stable than

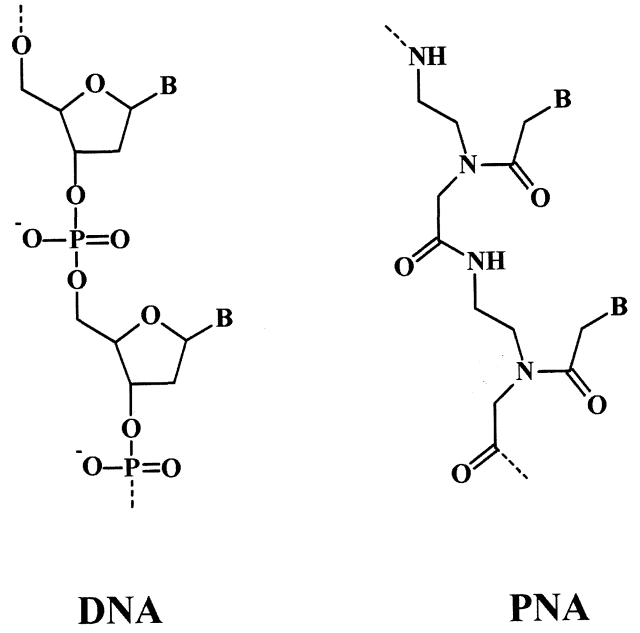

FIG. 1. Chemical structures of DNA and PNA. B is a nucleobase (adenine, guanine, cytosine or thymine).

the corresponding DNA complexes, and the sequence discrimination of the complexness is at least as good as that of DNA (6). Thus PNA form Watson-Crick base-pairing duplexes with complementary DNA or RNA.

The circular dichroism spectrum of the PNA–DNA duplex was found to be very close to that of a normal DNA–DNA duplex indicating that the PNA–DNA duplex has a B-DNA like helix conformation (6). This conclusion has been confirmed by proton NMR structure analyses (10). Interestingly a PNA–RNA duplex exhibits an A-RNA like helix conformation (2).

We have also measured the thermodynamic parameters for the PNA–DNA and the DNA–DNA duplexes. Focusing on the change in entropy (ΔS) upon duplex formation, we observed that this is very similar for the two duplexes. One might expect the backbone in PNA to be more flexible than the DNA backbone, and when converted from a flexible single-stranded to a much more rigid double-stranded structure much entropy is to be lost because of this flexibility, but apparently this is not the case. PNA appears to have a high degree of order, even in the single-stranded state. Thus, thermodynamically, PNA behaves very much like DNA (6).

The increased stability of the PNA–DNA duplex is primarily due to the lack of charge on the PNA backbone; because it is a neutral backbone, there is no repulsion between the backbones in the PNA–DNA duplex. While the thermal stability of DNA duplexes is highly salt-dependent (at low salt concentrations, DNA duplexes are much less stable than at high salt concentrations), PNA–DNA duplex

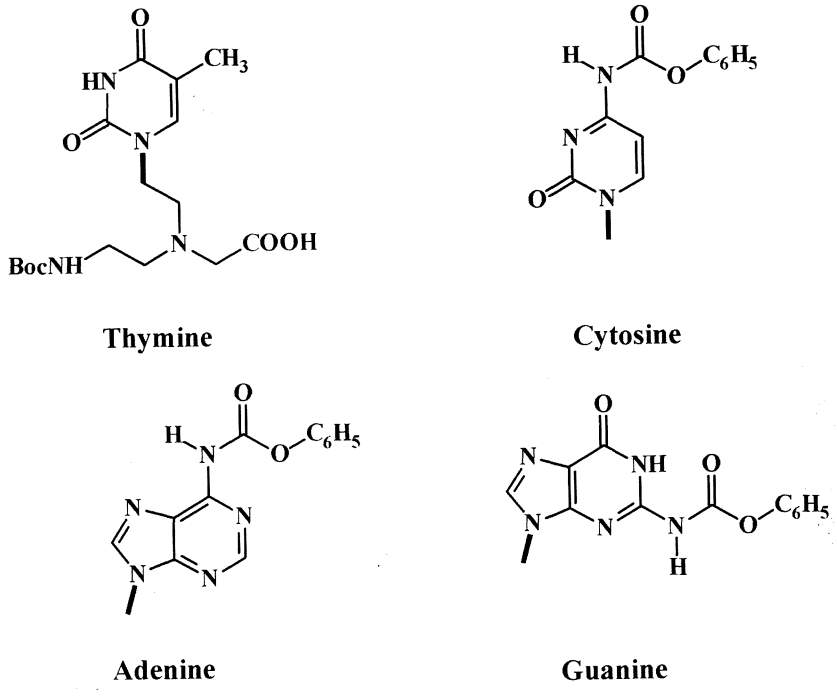

FIG. 2. Chemical structures of protected adenine, guanine, cytosine and thymine PNA monomers for solid support Boc-type synthesis of PNA oligomers.

stability shows very little salt dependence, and PNA–DNA and DNA–DNA duplex stabilities converge around 1 M salt (6).

PNAs consisting of only pyrimidines (cytosines and thymines) form triplexes with complementary DNA (7, 9). These triplexes consist of one DNA and two PNA strands employing Watson-Crick and Hoogsteen base-pairing recognition (14), and they are extremely stable. In fact, they are so stable that if a double-stranded DNA target is challenged with such a PNA, instead of binding to the surface of the DNA in a conventional triplex structure, the PNA displaces one DNA strand and hybridizes to the complementary strand. That produces a very stable complex in which two PNA strands clamp a single DNA strand (3, 13, 14). If the target is long enough, this structure can be seen by electron microscopy (3).

What can we learn from PNA in terms of prebiotic life? The first question concerns the "structural window" of the backbone; i.e., how much can the backbone be altered and still result in a structure that will mimic DNA? To date only few alterations have been reported. One of these is by extending the backbone by inserting an extra methylene group, yielding a β-alanine-based backbone (Fig. 3). We also made a PNA where the ethyl group was extended to a propyl group or the acetic acid was extended to yield a propionic acid. Thus we have extended the backbone in all three directions. These alterations

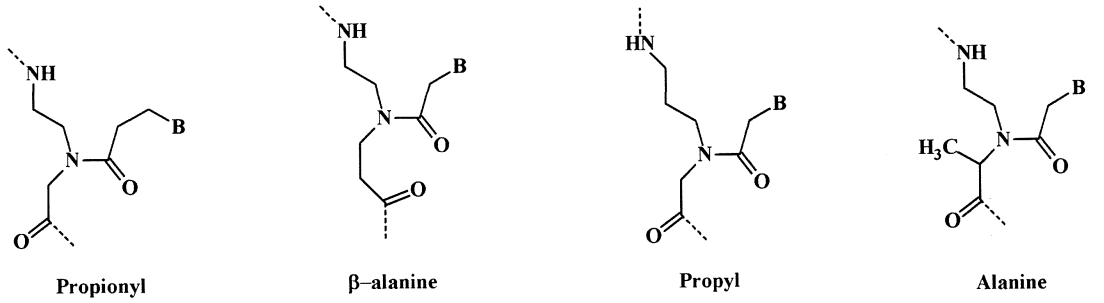

FIG. 3. Backbone modified PNA units.

destroy most of the DNA binding activity. Indeed, if you make PNA decamers consisting of units with backbones of only β-alanine, propyl, or propionic acid, they do not bind efficiently to DNA. However, even if these fully modified PNAs do not bind to DNA, PNAs with only a single unit to sequence specifically recognize DNA (8).

In a prebiotic context one should keep in mind that a primordial genetic material should not form duplexes of too high stability since the replication process necessarily involves strand separation. Thus there is a balance between "instability" and recognition.

Prebiotic relevance of PNA

In terms of prebiotic genetic material, formation of a stable PNA–PNA duplex would be required. Comparing the thermal stabilities of DNA–DNA and PNA–PNA complexes, we observe that the PNA–PNA complex is very stable; it is much more stable than the DNA–DNA and also than the PNA–DNA duplex (17). Titration experiments monitored by circular dichroism (CD) furthermore revealed a 1:1 ratio, consistent with a duplex structure. Since the backbone is achiral it might seem surprising that these PNA duplexes produce a CD signal. However, the chirality of the helix in these PNAs is induced by the attached lysine at the end (Fig. 4). Mixing two PNAs, each with a lysine at the end, results in a very nice CD spectrum, indicating that the complex is a helical duplex with a preferred orientation (17). Furthermore, this CD spectrum is very close to that of a B-DNA duplex, strongly indicating a similar helical structure for the PNA–PNA duplexes.

In principle, chiral seeding could also be introduced externally by binding the PNA duplex to some type of chiral surface, yielding a chiral supermolecule. Thus this principle could help explain the homochirality of life on Earth (11, 16).

Even though it has been indicated that the aminoglycine PNA backbone could be a prebiotic molecule, other possibilities may be more relevant. Some alternative suggestions for prebiotic PNA monomers are based on diaminobutyric acid or on ornithin (11) (Fig. 5).

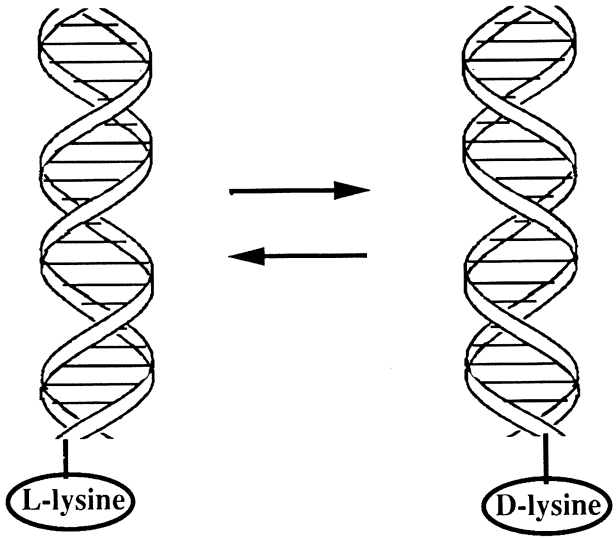

FIG. 4. Induced chirality of PNA double helices.

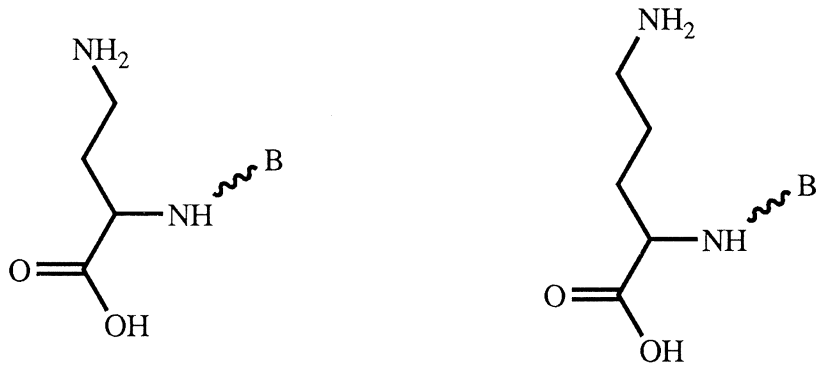

FIG. 5. Possible prebiotic PNA monomers.

In terms of prebiotic synthesis it is noteworthy that a photochemical reaction for exchanging the connection point of the pyrimidines to any ligand was discovered more than 10 years ago (15). If a nucleoside or an N-substituted pyrimidine is mixed with another amine and irradiated, an exchange of the amines results (Fig. 6). This reaction could thus attach pyrimidines to any type of amino acid.

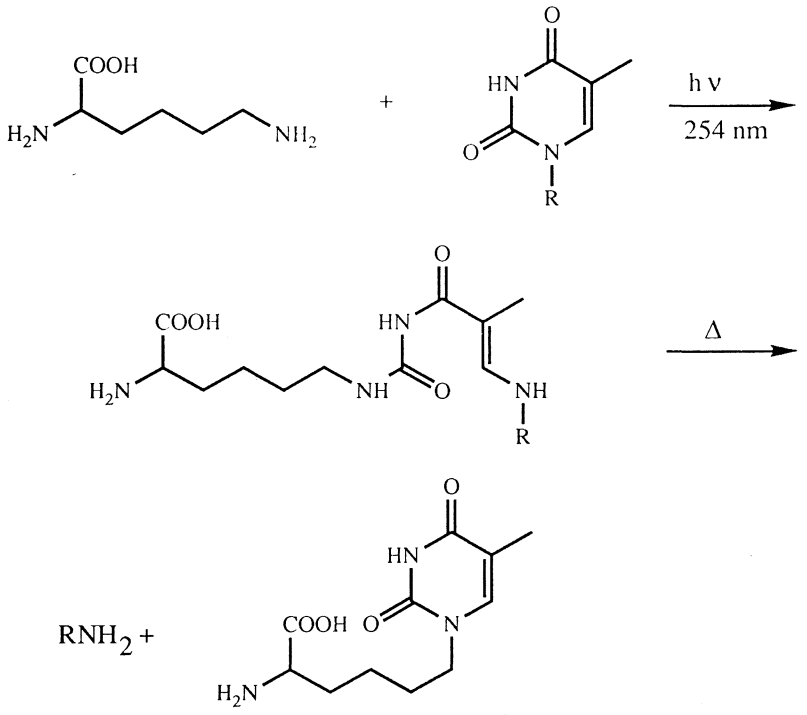

FIG. 6. A photochemical pyrimidine exchange reaction (16).

"PNA world"

If we speculate that there was some type of a "PNA-like world", we would require a transition from that world to our present-day world, a phosphate-based world. Thus it is important that PNAs form duplexes both with themselves and with normal nucleotides. One can imagine a transition involving a PNA template and RNA precursors from which a PNA–RNA duplex can be synthesized. Indeed, new results have demonstrated that both PNA template-directed chemical synthesis of RNA and RNA template-directed chemical synthesis of PNA are possible (16). It could be hypothesised that a sequence having catalytic RNA activity could evolve. If one accepts that the catalytic activity of the ribosome resides in the RNA, one can imagine having a catalytic RNA that is able to make the peptide bonds in the peptide-like molecule.

Studies of PNA have shown that information-bearing (genetic) material does not necessarily have to contain a phosphodiester-like backbone, but could, as illustrated by PNA, contain a pseudo-peptide (polyamide) backbone. This opens the possibility that the primordial genetic material could have been an achiral polyamide (analogous to PNA), perhaps even containing nucleobases different from

(and fewer than) the ones used by living organisms today. Later in evolution a (chiral) transition to an RNA world and further to the present day protein/RNA/DNA world could have taken place.

I do not wish to claim that PNA is a prebiotic structure, however I believe that the results obtained with PNA forces us to have an open mind to the chemical structure of a primordial genetic material.

References

1. Böhler, C., Nielsen, P. E., Orgel, L. E. (1995). Template switching between PNA and RNA. Nature, in press.
2. Brown, S. C., Thomson S. A., Veal, J. M., Davis, D. G. (1994). NMR solution structure of a peptide nucleic acid complexed with RNA. Science **265**, 777–780.
3. Chemy, D. Y., Belotserkovskii, B. P., Frank-Kamenetskii, M. D., Egholm, M., Buchardt, O., Berg, R. H., Nielsen, P. E. (1993). DNA unwinding upon strand displacement of binding of PNA to double stranded DNA. Proc. Natl. Acad. Sci. USA **90**, 1667–1670.
4. Christensen, L., Fitzpatrick, R., Gildea, B., Petersen, K. H., Hansen, H. F., Koch, T., Egholm, M., Buchardt, O., Nielsen, P. E., Coull, J., Berg, R. H. (1995). Solid-phase synthesis of peptide nucleic acids (PNA). J. Peptide Sci. **3**, 175–183.
5. Dueholm, K. L., Egholm, M., Behrens, C., Christensen, L., Hansen, H. F., Vulpius, T., Petersen, K., Berg, R. H., Nielsen, P. E., Buchardt, O. (1994). Synthesis of peptide nucleic acid monomers containig the four natural nucleobases: thymine, cytosine, adenine and guanine, and their oligomerization. J. Org. Chem. **59**, 5767–5773.
6. Egholm, M., Buchardt, O., Christensen, L., Behrens, C., Freier, S. M., Driver, D. A., Berg, R. H., Kim, S. K., Norden, B., Nielsen, P. E. (1993). PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen bonding rules. Nature **365**, 556–568.
7. Egholm, M., Buchardt, O., Nielsen, P. E., Berg, R. H. (1992). Peptide nucleic acids (PNA). Oligonucleotide analogues with an achiral peptide backbone. J. Amer. Chem. Soc. **114**, 1895–1897.
8. Hyrup, B., Eghlom, M., Nielsen, P. E., Wituung, P., Nordén, B., Buchardt, O. (1994). Structure-activity studies of the binding of modified peptide nucleic acids (PNA) to DNA. J. Amer. Chem. Soc. **116**, 7964–7970.
9. Kim, S. K., Nielsen, P. E., Egholm, M., Buchardt, O., Berg, R. H., Norden, B. (1993). Right-handed triplex formed between peptide nucleic acid PNA-T8 and poly(dA) shown by linear and circular dichroism spectroscopy. J. Amer. Chem. Soc. **115**, 6477–6481.
10. Leijon, M., Graslund, A., Nielsen, P. E., Buchardt, O., Norden, B., Kristensen S. M., Ericsson, M. (1994). Structural characterization of PNA-DNA duplexes by NMR. Evidence for DNA in a B-like conformation. Biochemistry **33**, 9820–9825.
11. Nielsen, P. E. (1993). Peptide nucleic acid (PNA). A model structure for the primordial genetic material. Orig. Life Evol. Bios. **23**, 323–327.
12 Nielsen, P. E. (1995). DNA analogues with nonphosphodiester backbones. Annu. Rev. Biophys. Biomol. Struct. **44**, 167–183.
13. Nielsen, P. E., Egholm, M., Berg, R. H., Buchardt, O. (1991). Sequence selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science **254**, 1497–1500.
14. Nielsen, P. E., Egholm, M., Buchardt, O. (1994). Evidence for $(PNA)_2$/ DNA triplex structure upon binding of PNA to dsDNA by strand displacement. J. Mol. Recognition **7**, 165–170.
15. Saito, I., Matsuura, T. (1985). Chemical aspects of UV-induced crosslinking of proteins to nucleic acids. Photoreactions with lysine and tryptophan. Acc. Chem. Res. **18**, 134–141.
16. Schwartz, A. W. (1994). The origin of macromolecular chirality. Curr. Biol. **4**, 758–760.
17. Wittung, P., Nielsen, P. E., Buchardt, O., Egholm, M., Norden, B. (1994). Double helix formation by complementary PNA. Direct observation of helical seeding. Nature **368**, 561–563.

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Insights to primitive replication derived from structures of small oligonucleotides

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Summary

Available information on the structure of small oligonucleotides is surveyed. It is observed that even small oligomers typically exhibit defined structures over a wide range of pH and temperature. These structures rely on a plethora of non-standard base-base interactions in addition to the traditional Watson-Crick pairings. Stable duplexes, though typically antiparallel, can be parallel or staggered and perfect complementarity is not essential. These results imply that primitive template directed reactions do not require high fidelity. Hence, the extensive use of Watson-Crick complementarity in genes rather than being a direct consequence of the primitive condensation process, may instead reflect subsequent selection based on the advantage of accuracy in maintaining the primitive genetic machinery once it arose.

Key words: prebiotic replication, oligonucleotide structure, base pairing, mismatch duplexes, DNA structure

Resumen

En este artículo se analiza la información disponible sobre la estructura de pequeños oligonucleótidos. Se ha observado que incluso los pequeños oligómeros presentan normalmente estructuras definidas en un amplio rango de pH y temperatura. Estas estructuras, además de en los apareamientos tradicionales

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de Watson-Crick, dependen de una plétora de interacciones base con base no estándar. Los duplexos estables, aunque sean típicamente antiparalelos, también pueden ser paralelos o solaparse, lo que nos indica que la complementariedad perfecta no es esencial. Estos resultados implican que reacciones primitivas dirigidas por un molde no requieren una elevada fidelidad. De esta manera, el uso extensivo de la complementariedad de Watson-Crick en los genes no es consecuencia directa de un proceso primitivo de condensación, sino que refleja, tras su aparición, la selección posterior basada en la ventaja de tener una maquinaria genética precisa.

Introduction

The emergence of template-directed replication is widely believed to be a key event in the origin of life. Hence, a variety of studies have focused on non-enzymatic template-directed synthesis of DNA and RNA oligomers under plausible prebiotic conditions. In pioneering work on RNA, by Orgel and others, synthesis was found to require polypyrimidine (especially, cytosine) rich templates to obtain reasonable yields (12, 20). The use of thymidine templates is restricted by the fact that adenine has relatively poor incorporation rates due to an intrinsically less-efficient condensation reaction (12). Success with oligomerization using polypurine templates has also been limited (28).

These template limitations are a concern because even with a successful initial condensation, subsequent evolution would appear to require that a synthesized complementary strand act as a template for further rounds of replication. Difficulty in obtaining significant yields in non-enzymatic template directed synthesis likely relates to stable structure in the templates. For example, poly-GC templates have been found to form both inter-molecular duplex structures (13) and intra-molecular stem-hairpin structures. Oligomerization in such cases might only occur at single-stranded sites such as the loop structures or by displacement of the complementary strand of the template. It is thus clear that oligonucleotide structure is potentially of great significance in determining the outcome of prebiotic condensations. These results have stimulated us to review what is known about the structure of small oligonucleotides and to consider what the implications of these findings are for studies of the origins of life.

Base pairing

Recent results convincingly demonstrate that a world in which the only stable base pairing is Watson-Crick G–C and A–T (or U) simply does not exist. There are theoretically 28 ways in which the standard bases can form pairs that involve at least two hydrogen bonds (24). Many of these interactions have been encountered in known structures of large RNAs and DNAs. For example, yeast $tRNA^{Phe}$ (14, 15) has a G–U pair, a reverse Watson-Crick pair and a N1–N1 G–A pair. An N7-amino, amino-N3 A–G pair has been found in loop E of eukaryotic 5S rRNA (33) and the G–U reverse wobble pair has been found in an RNA tetraloop (30). Phylogenetic evidence has also suggested numerous nonstandard

pairings in the large rRNAs as well (7). The individual pairing possibilities are not restricted to these 28, but are actually further enhanced by the occurrence of triplet interactions, such as occur in tRNA (15), and the existence of water mediated base pairs, such as the N3–N3 4-carbonyl-amino C–U pair found by crystallography in a small RNA duplex (8). In addition, new pairing possibilities are opened up by protonation of adenine and cytosine at low pH.

Oligonucleotide structure

What is perhaps less well known, is that these same non-standard pairing motifs are also found in very small oligonucleotides. Thus, short RNA and DNA oligomers that would appear to form no secondary structure or, form structures that would appear significantly less stable than complementary duplexes, do in fact form stable structures at room temperature and at neutral pH. For example, one study utilized an 8mer (rCGCXXGCG, where X = [G, A, U, C]) to determine the relative stabilities of various two base pair mismatches in RNA internal loops (25). Although it was determined that there are two classes of internal loops, those that stabilize duplexes and have strong hydrogen bonding and those that destabilize the duplex and may not have strong hydrogen bonding, all the 8mers melted above 32°C at neutral pH. Some structures were stabilized by decreasing the pH. At pH 6 the T_m of the duplex containing an internal loop consisting of two C–C mismatches increases 12°C relative to pH 7. Ostensibly a C becomes protonated at the lower pH and hemi-protonated C^+–C base pairs form. This shows that mismatches, even those that destabilize duplex structure, can easily be incorporated into a relatively stable duplex and under certain conditions (altered pH, salt concentration, ion type) may stabilize the duplex. Mismatches between G and A have attracted considerable attention and it has been found that a G–A pair adjacent to an A–G pair is specially compatible with the usual B-form DNA duplex. Hence, the sequence d(ATGAGCGAAT) was found (17) to form a stable duplex despite the fact four of ten base pairs are not of the Watson-Crick type.

Protonation of cytosine can in particular be a driving force in the formation of alternative base pairing schemes because it precludes the formation of a typical Watson-Crick G–C base pair. Of all the bases, cytosine has the highest pK_a value of 4.2 (3). This pK, is, however, strongly influenced by structure and thus the pK_a of cytosine has been determined to be as low as 3.7 in calf thymus DNA (2) and as high as 7.4 in poly(dC) oligomers (10). Recently, it has been determined that a non-self-complementary DNA heptamer d(CGACGAC) can form an exclusively homo base-paired parallel duplex at acidic to near neutral pH that is stable to 40°C at the lower pH (23). The structure consists of a helix that possesses an exact two-fold symmetry coinciding with the helical axis. The strands are interdigitated and the groove is very shallow at the G–G base pair step. The stability of the parallel duplex is derived in part from the hemi-protonation of the cytosine residues which enable the formation of three C^+–C base pairs. The authors postulate that additional stability is derived from the interstrand G–A stacking interactions in which the A-over-A and G-over-G stack is energetically favored (6, 17). Additional evidence indicates that 5'–CGA may induce other sequences to form homo base-paired parallel-stranded DNA duplexes (22).

NMR and non-denaturing gel electrophoresis have been used to probe the three dimensional structure of several DNA triplet repeats: $(CTG)_n$, $(CAG)_n$, $(CTG)_n$–$(CAG)_n$, $(CCG)_n$, $(CGG)_n$, $(GAC)_n$, where the repeat number is between 2 and 5, as well as permutation sequences of varying length (Gao et al., personal communication). In almost every sequence examined to date a pattern consistent with inter-strand interaction appears. In the least extreme cases, sequences that approximate B-form DNAs with canonical Watson-Crick base-pairing interrupted by a base-pair mismatch every third base are found. Typically these duplexes are more stable than thermodynamic theory would predict. For example, $(CTG)_3$ forms an anti-parallel, B-form DNA duplex which is stable at temperatures approaching 30°C (27). The duplex backbone narrows nearly 1.5 Å to accommodate T–T pairing that appears to add the unexpected stability to the duplex. It is possible that water may mediate the T–T interaction. Even $(CTG)_2$ appears to form a duplex structure that is stable at low temperature. Although $(CAG)_2$ appears to form only random coil at low temperatures, the addition of only one repeat provides an anti-parallel duplex that is stable near room temperature. Longer $(CTG)_n$ repeats ($n > 15$) form stable elongated hairpin structures (18). In the case of $(CCG)_2$, a particularly unusual duplex structure is found. It consists of an anti-parallel duplex which is staggered by a single base with single "looped-out" bases that are actually accommodated into the minor groove of the duplex (4). When this repeat sequence is expanded to 3–10 repeats the unusual structure is maintained but finds itself in equilibrium with anti-parallel and parallel duplexes. In addition, d(CGCCG) forms a parallel duplex in 50/50 equilibrium with anti-parallel duplex at room temperature and neutral pH. Many longer repeat sequences demonstrate multiple conformations that exist in an equilibrium which in some cases can be altered by changing buffer conditions. In addition, certain repeat sequences, guanine-rich, appear to form higher order structures as evidenced by non-denaturing gel electrophoresis. All of these structures exist at temperatures close to room temperature and at nearly neutral pH.

DNA high order structures

Certain DNA sequences are capable of forming higher order structures, ones in which single DNA strands self-associate to form quadruplex structures. Exemplary of these structures are the i-motif (16) and the well known g-tetrad (31). The i-motif is characterized by a four-stranded complex in which parallel duplexes assemble in an anti-parallel fashion. Two base-paired parallel stranded duplexes are associated such that their base-pairs are fully intercalated. While the strand association is anti-parallel, the duplex association is parallel such that each base pair is face-to-face with its neighbors. This motif has been demonstrated in the hexamer sequence d(TCCCCC) at low pH (5). Other sequences such as d(CCCCAA) and d(CCCCAAAA) when analyzed by UV-melting, chemical modification and native gel electrophoresis have been reported to form compact structures in a wide pH range (1). Longer sequences such as $d([CCCTAA]_3CCC)$ representative of the telomeric regions in vertebrates, have also demonstrated this unique motif. Although pH dependent, these structures have melting transition temperatures above room temperature. Ostensibly formation of i-motif-like structures is driven by C^+–C base-pairing. Formation of the tetrad demonstrates concentration dependence. Above 100 μM the tetrad dominates

while at concentrations below 1 μM the single stranded species is the sole form. In concentrations between 1–100 μM a parallel duplex appears. The tetrad has several energetic advantages over the duplex: (i) the tetrad has 11 base-pair stacking interactions as opposed to 10 in the duplex, (ii) favorable electrostatic interactions resulting from C protonation and (iii) extensive sugar-sugar van der Waals interactions which are absent in the duplex (5).

Analogously, G-rich sequences have been found to make quartet-type structures. In the presence of monovalent cations, guanines can adopt a planar G-tetrad alignment. When incorporated into a linear sequence these structures are capable of stacking on one another to form G-tetraplexes (31). Tetraplexes stabilized by G-tetrads are formed by human $d(AG_3[T_2AG_3]_3)$, *Tetrahymena* $d(T_2G_4)_4$, (32) and *Oxytricha* $d(G_4T_4G_4)$ (26) telomeric repeats. The tetrads approach planarity in the tetraplex and stability is achieved through extensive stacking between five-membered rings of adjacent guanines. Although the global features of both tetraplexes involve three stacked G-tetrads the difference in the two sequences, an A substituted for G, causes profound changes in the folding topology. Although both structures are stable, substitution of different monovalent cations and bases (i.e., guanine → inosine) have drastic effects on stability.

Other higher order structures formed by DNA include H-DNA, which contains both triple and single stranded regions (9), and triplex DNA stabilized by T–AT, and C^+–GC triple base-pairs (21). Implicated in both of these structures are alternating $(C–T)_n$ sequences. The ability of this alternating sequence to form a variety of intra and inter-strand structures comes from its conformational polymorphism. There are three major conformational spec[illegible] (i) at low pH, an antiparallel stranded duplex with entirely C^+–T base pairs, (ii) at neutral pH, an anti-parallel stranded structure with C–T base-pairs, and (iii) at pH = 5, a parallel stranded structure comprised of C^+–C and T–T base-pairs (11).

Finally, RNA and DNA sequences of only modest length can form stable intra-strand interactions, namely hairpin loops at even very low concentrations. For example, the 12mer r(GGACUUCGGUCC) forms an especially stable tetraloop structure (30) and an only slightly longer sequence, r(GCGAUUUCUGACCGCC), forms a UCU triloop, with a six base-pair stem that includes A^+–C, and U–G pairings (19). The DNA 11mer, d(CGCGATTCGCG) can form either a hairpin structure or a duplex with a single T–T opposition, and equilibrium mixtures of both have been observed (29).

Conclusions

This brief overview of a rapidly growing literature reveals that RNA and DNA oligonucleotides of modest size (6–15 residues) can produce a surprising variety of structures that are stable over a wide range of pH and temperature. What lessons for prebiotic chemistry can be derived from these results? Probably the most noteworthy result is that stable duplex structures can definitely occur despite considerable "mispairing". Clearly the existence of structure in even small oligomers needs to be anticipated in the design of experiments relating to non-enzymatic replication. That this has not been overlooked is indicated by a recent study (34), in which hairpin loop structures have been used to produce non-enzymatic condensations at very low template concentrations with well defined initiation sites.

The fact that even very small oligonucleotides can have significant structural stability may offer

some novel insights into the origin of life. Thus, it is immediately clear that a template-directed condensation may be able to continue without the new strand falling off despite the generation of mismatches. Mismatched duplexes may even be advantageous during the early stages of prebiotic synthesis as they may be more amenable to strand separation than fully paired duplexes.

The need for accuracy of replication in modern organisms reflects the use of nucleic acids as genetic material. At the stage in the origin of life where primitive replication first began, the genetic machinery likely did not yet exist. From the perspective of current thinking, the most essential role of the initial replication system would be to build up RNAs (or RNA analogs) of sufficient size that catalytic nucleic acids could be generated. Accuracy would not matter initially (unless it is essential for the condensation) for the simple reason that there was no genetic information to store. If, however, a primitive ribozyme capable of catalyzing the synthesis of peptides were to arise then peptides which improved the accuracy and/or speed of replication would likely have an advantage in that the ribozymes responsible for their synthesis would have a better chance of surviving. Hence, in this scenario, the genetic machinery would essentially co-evolve and translation/replication would be essentially equally ancient. Alternatively, as it is currently being pursued by several groups, the first useful ribozyme might have itself been a primitive replicase. From this perspective, replication would likely predate translation.

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References

1. Ahmed, S., Henderson, E. (1992). Formation of novel hairpin structures by telomeric C-strand oligonucleotides. Nucl. Acids Res. **20**, 507–511.
2. Banville, D. L., Marzilli, L. G., Wilson, W. D. (1986). NMR Investigation of DNA conformational changes on base protonation: use of Cu^{2+} and pyrazole as probes. Biochemistry **25**, 7393–7401.
3. Bloomfield, V., Crothers, D. M., Tinoco Jr., I. (1974). *In* Physical Chemistry of Nucleic Acids, pp. 332–371. Harper & Row, New York, NY.
4. Gao, X., Huang, X., Smith, G. K., Zheng, M., Liu, H. (1995). A new antiparallel duplex motif of DNA that is stabilized by extrahelical bases symetrically located in the minor groove, in press.
5. Gehring, K., Leroy, J.-L., Gueron, M. (1993). A tetrameric DNA structure with protonated cytosine-cytosine base pairs. Nature **363**, 561–565.
6. Greene, K. L., Jones, R. L., Li, Y., Robinson, H., Wang, A. H.-J., Zon, G., Wilson, W. D. (1994). Solution structure of a GA mismatch DNA sequence $d(CCATGAATGG)_2$ determined by 2D NMR and structural refinement methods. Biochemistry **33**, 1053–1062.

7. Gutell, R. (1993). Comparative studies of rRNA: inferring higher-order structure from patterns of sequence variation. Curr. Opi. Struct. Biol. **3**, 313–322.
8. Holbrook, S. R., Cheong, C., Tinoco Jr., I., Kim, S.-H. (1991). Crystal structure of an RNA double helix incorporating a track of non-Watson-Crick base pairs. Nature **353**, 579–581.
9. Htun, H., Dahlberg, J. E. (1988). Single strands, triple strands, and kinks in H-DNA. Science **241**, 1791–1795.
10. Inman, R. B. (1964). Transitions of DNA homopolymers. J. Mol. Biol. **9**, 624–637.
11. Jaishree, T. N., Wang, A. H.-J. (1993). NMR studies of pH-dependent conformational polymorphism of alternating $(C–T)_n$ sequences. Nucleic Acids Res. **21**, 3839–3844.
12. Joyce, G. F., Inoue, T., Orgel, L. E. (1984). Non-enzymatic template directed synthesis on RNA random copolymers: poly (C, U) templates. J. Mol. Biol. **176**, 279–306.
13. Joyce, G. F., Orgel, L. E. (1986). Non-enzymic template-directed synthesis on RNA random copolymers: poly (C, G) templates. J. Mol. Biol. **188**, 433–441.
14. Kim, S.-H. (1978). Crystal structure of yeast $tRNA^{Phe}$: its correlation to the solution structure and functional implications. *In* Altman, S. (ed.), Transfer RNA, pp. 248–293. MIT Press, Cambridge, MA.
15. Kim, S.-H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wang, A., Seeman, N. C., Rich, A. (1974). Science **185**, 435–440.
16. Leroy, J.-L., Gueron, M., Mergny, J.-L., Helene, C. (1994). Intramolecular folding of a fragment of the cytosine-rich strand of telomeric DNA into an i-motif. Nucleic Acids Res. **22**, 1600–1606.
17. Li, Y., Zon, G., Wilson, W. D. (1991). Thermodynamics of DNA duplexes with adjacent G–A mismatches. Biochemistry **30**, 7566–7572.
18. Mitas, M., Yu, A., Dill, J., Kamp, T. J., Chambers, E. J., Haworth, I. S. (1995). Hairpin properties of single stranded DNA containing a G–C rich triple repeat. Nucleic Acids Res. **23**, 1050–1059.
19. Puglisi, J. D., Wyatt, J. R., Tinoco Jr., I. (1990). Solution conformation of an RNA hairpin loop. Biochemistry **29**, 4215–4226.
20. Ng, K.-M. E., Orgel, L. E. (1989). Reaction on alternating GC oligonucleotide templates. J. Mol. Evol. **29**, 101–107.
21. Radhakrishnan, I., Patel, D. J. (1994). Solution structure of a pyrimidine-purine-pyrimidine DNA triplex containing T–AT, C^+–GC and G–TA triples. Structure **2**, 17–32.
22. Robinson, H., van Boom, J. H., Wang, A. H.-J. (1994). 5'–CGA motif induces other sequences to form homo base-paired parallel-stranded DNA duplex: the structure of $(G–A)_n$ derived from four DNA oligomers containing $(G–A)_3$ sequence. J. Am. Chem. Soc. **116**, 1565–1566.
23. Robinson, H., Wang, A. H.-J. (1993). 5'–CGA sequence is a strong motif for homo base-paired parallel-stranded DNA duplex as revealed by NMR analysis. Proc. Natl. Acad. Sci. USA. **90**, 5224–5228.
24. Saenger, W. (1984). Principles of Nucleic Acid Structure. Springer-Verlag, New York, NY.
25. SantaLucia Jr., J., Kierzek, R., Turner, D. H. (1991). Stabilities of consecutive A–C, C–C, G–G, U–C, and U–U mismatches in RNA intemal loops: Evidence for stable hydrogen-bonded U–U and C^+–C pairs. Biochemistry **30**, 8242–8251.
26. Schultz, P., Smith, F. W, Feignon, J. (1994). Refined solution structure of the dimeric quadraplex formed from *Oxytricha* telomeric oligonucleotide d(GGGGTTTTGGGG). Structure **2**, 221–233.
27. Smith, G. K., Jie, J., Fox, G. E., Gao, X. (1995). DNA CTG repeats involved in dynamic mutations of neurologically related gene sequences form stable duplexes, in press.
28. Stribling, R., Miller, S. L. (1991). Attempted nonenzymatic template-directed oligomerizations on a polyadenylic acid template: Implications for the nature of the first genetic material. J. Mol. Evol. **32**, 282–288.
29. Summers, M. F., Byrd, R. A., Gallo, K. A., Samson, C. J., Zon, G., Egan, W. (1985). Nuclear magnetic resonance and circular dichroism studies of a duplex-single-stranded hairpin loop equilibrium for the oligodeoxyribonucleotide sequenced (CGCGATTCGCG). Nucl. Acids Res. **13**, 6375–6386.

30. Varani, G., Cheong, C., Tinoco Jr., I. (1991). Structure of an unusually stable RNA hairpin. Biochemistry **30**, 3280–3289.
31. Wang, Y., Patel, D. J. (1993). Solution structure of the human telomeric repeat d[$AG_3(T_2AG_3)_3$]G-tetraplex. Structure **1**, 263–282.
32. Wang, Y., Patel, D. J. (1994). Solution structure of the *Tetrahymena* telomeric repeat d(T_2G_4) G-tetraplex. Structure **2**, 1–17.
33. Wimberly, B., Varani, G., Tinoco Jr., I. (1993). Conformation of loop E from eukaryotic 5S rRNA. Biochemistry **32**, 1078–1087.
34. Wu, T., Orgel, L. E. (1992). Nonenzymatic template-directed synthesis on oligodeoxycytidylate sequences in hairpin oligonucleotides. J. Am. Chem. Soc. **118**, 317–322.

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The reverse gyrase of hyperthermophilic archaeobacteria: origin of life and thermophily

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Summary

Several authors have suggested that life originated at high temperature and that present hyperthermophilic prokaryotes, especially archaeobacteria are representatives of ancient life forms. A unique DNA topoisomerase, reverse gyrase, is present in all hyperthermophilic organisms studied so far. Plasmids isolated from archaeobacteria with reverse gyrase activity are relaxed, i.e. they have a higher number of topological links compared to negatively supercoiled plasmids from eubacteria and mesophilic archaeobacteria. Thus reverse gyrase is most probably required for DNA stabilization at very high temperatures. The gene encoding the reverse gyrase of the sulfothermophilic archaeobacterium *Sulfolobus acidocaldarius* has been recently cloned and sequenced. This sequence turns out to be a combination of a helicase gene and a DNA topoisomerase gene. This unique structure suggests that reverse gyrase originated after the appearence of helicase and topoisomerase, i.e. by the fusion of a DNA helicase gene and a DNA topoisomerase gene. Phylogenetic analyses of DNA polymerases and topoisomerases I and II amino acid sequences indeed suggest the existence of a specific evolutionary period between the first organism with a DNA genome and the last common ancestor of eubacteria, archaeobacteria and eukaryotes, a first age of the DNA world. The fact that reverse gyrase does not seem to be a primitive enzyme raises doubt about the existence of a direct link between a hot origin of life, hyperthermophilic prokaryotes and the universal ancestor.

Key words: topoisomerase, reverse gyrase, hyperthermophilic, DNA world, *Sulfolobus*

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Resumen

Varios autores defienden la idea de que las temperaturas altas propiciaron el desarrollo de la vida y que los procariotas hipertermofílicos actuales, especialmente las arqueobacterias, son representantes de antiguas formas de vida. Una DNA topoisomerasa especial, la girasa inversa, se encuentra en todos los organismos hipertermofílicos estudiados. Los plásmidos aislados de arqueobacterias con actividad de girasa inversa están relajados, es decir, tienen un número elevado de enlaces topológicos en comparación con los plásmidos superenrollados negativamente de eubacterias y arqueobacterias mesofílicas. La girasa inversa es necesaria para la estabilización del DNA a altas temperaturas. Recientemente, a partir de la arqueobacteria sulfotermofílica *Sulfolobus acidocaldarius,* hemos clonado y secuenciado el gen que codifica la girasa inversa. La secuencia resulta ser una combinación de un gen de una helicasa y un gen de una DNA topoisomerasa. Esta secuencia singular sugiere que la girasa inversa se originó después de la aparición de la helicasa y de la topoisomerasa, es decir, mediante la fusión de un gen de una DNA helicasa y de un gen de una DNA topoisomerasa. Los análisis filogenéticos de las secuencias de aminoácidos de las DNA polimerasas y de las topoisomerasas I y II, sugieren claramente la existencia de un período evolutivo específico comprendido entre el primer organismo con genoma de DNA y el último antepasado común de eubacterias, arqueobacterias y eucariotas, es decir, la primera edad del mundo DNA. El hecho de que la girasa inversa no parezca una enzima primitiva pone en duda la existencia de una relación directa entre un origen "caliente" de la vida, los procariotas hipertermofílicos y un antepasado universal.

Reverse gyrase

This article will not address directly the problem of the origin of life at high temperature, but it deals with the problem of a direct connection between a preadaptive origin of life at high temperature and the hyperthermophilic organisms. Many other investigators argue for the existence of such a direct link (7, 8). If we follow this reasoning, we should conclude that in some respect hyperthermophiles are at present among the most primitive organisms around us. One way to check the feasibility of this idea is to study these organisms at the molecular level and try to determine if they have specific mechanisms to live in high temperature environments, and if these mechanisms can be considered primitive.

The topic of this article, which focuses on recently published data, is an enzyme that we discovered about eight years ago, with a Japanese group, that can teach us about this problem. This enzyme, called reverse gyrase, has been found in the hyperthermophilic archaeobacterium *Sulfolobus acidocaldarius* (for a review on reverse gyrase and DNA supercoiling, see ref. 4). Reverse gyrase has the ability to introduce positive superturns into a DNA molecule (Fig. 1). Supercoiling of the DNA means that the double helix of the DNA is coiled around itself. If this coiling occurs in the same direction as the double helix itself, it is referred to as positive supercoiling. In such a DNA, the number of links between the two strands of the molecule is increased compared to a DNA that is relaxed.

The discovery that reverse gyrase can introduce positive superturns was very surprising because until then all the DNAs that had been isolated in nature, either from bacteria or from eukaryotic cells,

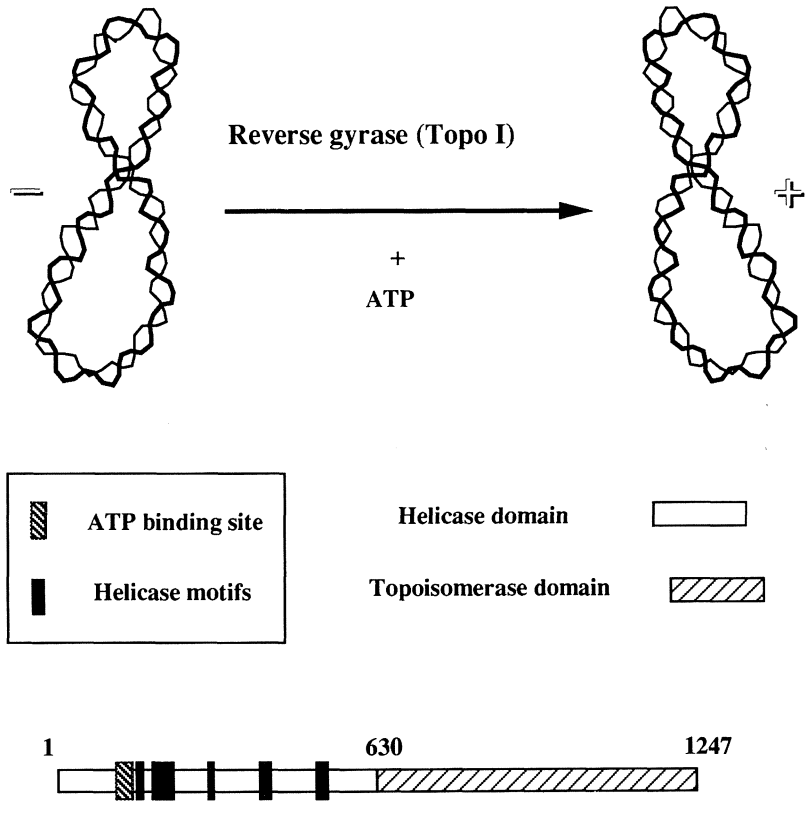

FIG. 1. Reverse gyrase activity (positive supercoiling of the DNA) and structure of its gene.

had been found to be negatively supercoiled. In negatively supercoiled DNA, the superhelix is coiled in the opposite direction to that of the double helix of Watson and Crick. This arrangement reduces the number of links between the two strands of the molecule. Thus, in a negatively supercoiled DNA, the double helix has a spontaneous tendency to open itself. Therefore, negative supercoiling helps all the mechanisms that require transient unwinding of the DNA molecule, such as DNA replication, transcription, and so on. The opposite is true of a positively supercoiled DNA: opening the double helix is very difficult. The discovery of reverse gyrase in a hyperthermophilic organism thus immediately led to the suggestion that this enzyme helps stabilize the DNA of the organism at high temperatures.

I will now present just a bit of DNA topochemistry and explain how we can distinguish between the action of an enzyme that produces positive superturns and the action of an enzyme, such as the previously known bacterial gyrase, that introduces negative superturns. The name reverse gyrase was derived as an opposite of bacterial gyrase, which was first discovered in *Escherichia coli*. Let me remind you that it is possible to distinguish between a supercoiled DNA and a relaxed DNA simply by running this molecule in an agarose gel, because supercoiled DNA migrates faster than relaxed DNA. DNAs with one

superturn, two superturns, three superturns, and so on are identical, except for the number of links between the two DNA strands. Such DNAs are called topoisomers, and the enzymes, such as gyrase and reverse gyrase, that can change the linking number between the two strands of the molecule are called topoisomerases. A simple agarose gel, however, tells you only how many superturns there are, not whether they are positive or negative.

To determine whether the superturns are negative or positive, we must run a 2-D gel using agarose gel electrophoresis (Fig. 2). During the second dimension of the electrophoresis, we add a drug that changes the supercoiling of the molecule. The drug intercalates into the double helix, thereby increasing the pitch of the molecule; then it reduces the number of turns in a DNA of fixed length. To compensate for this reduction in the number of turns, the drug introduces positive supercoiling into the DNA. Thus, for example, if you add a drug concentration that introduces five positive superturns into the DNA, a

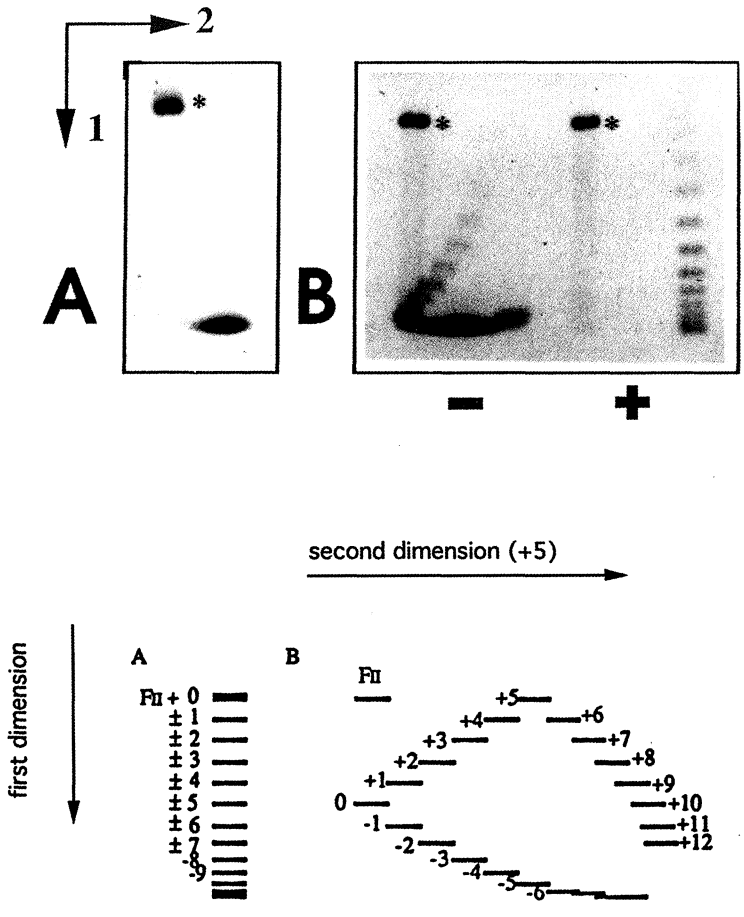

FIG. 2. Test of reverse gyrase activity: upper panel, detection of reverse gyrase activity on two-dimensional agarose gel; lower panel, principle of two dimensional agarose gel (see text for explanations).

topoisomer that has five negative superturns in the first dimension will have zero superturns in the second dimension, and a topoisomer with five positive superturns in the first dimension will have ten positive superturns in the second dimension. The latter will therefore migrate more rapidly than the former in the second dimension, enabling us to distinguish easily between positively and negatively supercoiled DNA.

The upper panel of Fig. 2, shows a test for reverse gyrase activity using a two-dimensional agarose gel. The control DNA substrate (a negatively supercoiled *Escherichia coli* plasmid) is shown in A. The same plasmid incubated with reverse gyrase (panel B) is only slightly relaxed in the absence of ATP (–) but highly positively supercoiled in the presence of ATP (+), showing that reverse gyrase is an ATP-dependent enzyme.

Several laboratories have looked for reverse gyrase in many different organisms. This enzyme has been found in all hyperthermophilic organisms studied so far, both archaeobacteria and bacteria, and in some thermophilic organisms with an optimal growth temperature of at least 75°C. We have looked for reverse gyrase in many bacteria, including mesophilic archaeobacteria, and moderately thermophilic bacteria, and have not found it. At the moment, then, there is a strong correlation between the presence of reverse gyrase and thermophily of the strain. This evidence does not prove that reverse gyrase is required by life at high temperatures, but we lack definitive proof mainly because the genetics of these organisms are not yet understood, i.e., we do not have mutants of the enzyme.

Recently, we have shown that organisms with a reverse gyrase activity have relaxed plasmids, compared to those with gyrase activity which have negatively supercoiled ones (1). This indicates that reverse gyrase indeed influences the overall DNA topology in vivo.

If we superimpose our data with reverse gyrase on the typical tree, based on ribosomal RNA sequence, reverse gyrase appears to be present in all the thermophilic lines, and absent in the others. If the tree is correctly rooted, then reverse gyrase must have been present in the common universal ancestor of all living organisms. I will return to this point, however, because we are not sure about the location of this root. In a certain sense we can now ask: is reverse gyrase really a primitive enzyme? If this scheme is correct—if we have a direct connection between the origin of life and the last common ancestor, which was a thermophilic organism—reverse gyrase should be a primitive enzyme. Thus, we would like to know more about it.

DNA topoisomerases are classified into two types: type I DNA topoisomerases, which introduce transient single-stranded breaks into DNA molecules; and type II DNA topoisomerases, which introduce transient double-stranded breaks into the DNA molecule. At first, reverse gyrase was thought to be a type II enzyme because all type II enzymes are ATP-dependent, whereas type I enzymes are ATP-independent. When we start to look at the mechanisms of reverse gyrase, however, we see that it is a type I enzyme. The purification scheme shows that purified reverse gyrase is a monomer, as are other type I enzymes, and that it produces a transient single-stranded break into the DNA, as type I enzymes do.

Recently we produced an antibody against this purified polypeptide, and used the antibody to clone the gene coding for reverse gyrase, revealing the structure of the enzyme (2). The result was very surprising: reverse gyrase appears to be a combination of two other enzymes. The C-terminal domain of the reverse gyrase aligns very well with other type I DNA topoisomerases, such as the ω protein in *E. coli*, the family of type I DNA topoisomerases that are known to relax only negative superturns in the

DNA. The N-terminal domain of reverse gyrase, however, is very unusual for a DNA topoisomerase. In fact, when we looked at a sequence, we found different amino-acids motives that are normally present only in DNA helicases (Fig. 1). DNA helicases are enzymes that do not introduce breaks into the DNA, but just separate the two strands of the double helix. In particular, reverse gyrase has an ATP-binding site, which is characteristic of the helicases and many families of ATPases. This binding site explains why reverse gyrase is ATP-dependent.

From this result, we can imagine a model for the activity of reverse gyrase (2). A few years ago it was shown that when an RNA polymerase moves along a DNA molecule, transcribing the DNA, it produces a wave of positive superturns in front of the molecule and a wave of negative superturns behind the molecule. More recently it was shown that a DNA helicase produces the same phenomenon. One can thus imagine that reverse gyrase moves along the DNA using its helicase activity to produce positive superturns in front of it and negative superturns behind. Since the topoisomerase domain of reverse gyrase belongs to a family of proteins that relax only negative superturns, there is a net accumulation of positive superturns by the process of helicase and topoisomerase activity.

Returning to our first question: reverse gyrase—is it old or new? Up to this point I have reported facts; now I am going to speculate. Different hypotheses are possible, but I think the most likely hypothesis is that reverse gyrase is a new enzyme that originated as a result of the fusion of a DNA topoisomerase and a DNA helicase (6). Thus, if reverse gyrase is required for life at high temperatures, we believe that organisms living before the fusion between the DNA helicase and DNA topoisomerase could not have lived at high temperatures. This finding thus argues against a direct connection to a putative hot origin of life, but we could imagine a period of evolution at relatively low temperature for the appearance of topoisomerase and helicase, followed by the appearance of reverse gyrase.

Fig. 3 shows two possible scenarios for the appearence of reverse gyrase in the early evolution of life. In the first scenario you can recognize the now quite classical tree relating the three domains, with the root of the tree in the bacterial branch (9). If we root the tree like that, and if we consider that the universal ancestor was a hyperthermophilic organism, reverse gyrase must have appeared before the universal ancestor did. Thus, one can ask, why was the universal ancestor a hyperthermophile? One possibility is what Dr. Gogarten suggested: that there was a catastrophe that killed all mesophilic organisms, leaving only the hyperthermophilic organisms as survivors.

However, the rooting of the tree of life in the bacterial branch is far from settled (3). So, many options are open. For example, one cannot dismiss the possibility that the root of the tree is in the eukaryotic branch, and reverse gyrase appeared in the lineage common to archaeobacteria and bacteria. There are two questions that are still important to solve: why are all hyperthermophiles prokaryotes? and why was the common ancestor of all prokaryotes an extreme thermophile? This second hypothesis is enhanced by the discovery of reverse gyrase in both hyperthermophilic bacteria and archaeobacteria.

Thus, I propose another hypothesis—that there is a direct connection between the appearance of prokaryotes and the adaptation to hyperthermophily—which explains why the common ancestor of all prokaryotes was a thermophile. The idea is that one of the main problems facing organisms living at very high temperatures is the degradation of the macromolecules, in particular the degradation of RNA. In fact, it is very convenient, when living at high temperature, to have a high micromolecular turnover, and in particular to prevent the nuclear membrane from using messenger RNA as soon as it is synthesized on the DNA (3). Thus, one possibility is that the universal ancestor were a mesophile that was neither

a eukaryote nor a prokaryote, and that prokaryotes appeared by thermoreduction from these organisms.

This hypothesis has a few advantages. It could explain, for example, why eukaryotes have some molecular mechanisms that appear to be remnants of the RNA world such as telomerases, editing, spliceosomes and so on. It also eliminates the need to explain how some of these prokaryotic organisms, which had not changed for 3.5 billion years, could suddenly have evolved into a eukaryote.

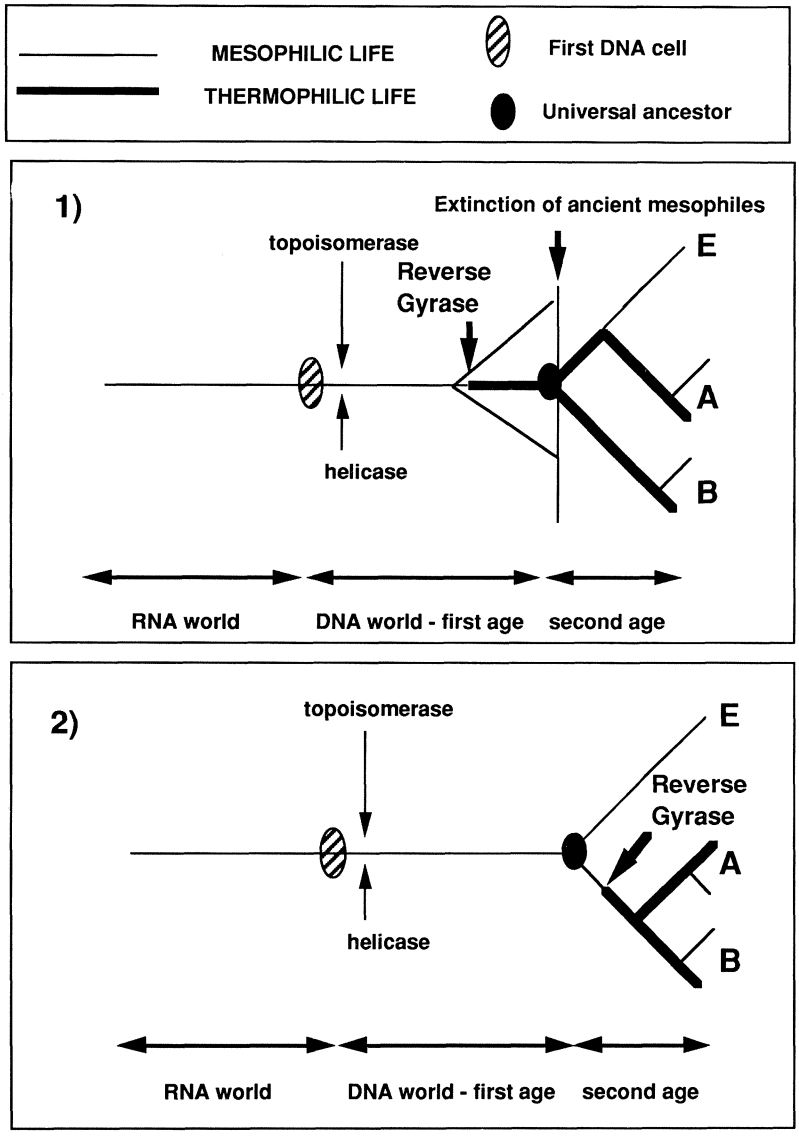

FIG. 3. Two scenarios for the early evolution of life and the origin of reverse gyrase. In both scenarios, the RNA world was mesophilic (5). Topoisomerases and helicases appeared in a first age of the DNA world (3), i.e. after emergence of the first DNA cell but before radiation of the three present cell lineages, *Archaea* (A), *Bacteria* (B) and *Eucarya* (E) from their last common ancestor. In scenario 1 the root of the tree of life is located in the bacterial branch (9). In scenario 2, it is located in the eukaryotic branch (5).

References

1. Charbonnier, F., Forterre, P. (1994). Comparison of plasmid DNA topology among mesophilic and thermophilic eubacteria and archaebacteria. J. Bacteriol. **176**, 1251–1259.
2. Confalonieri, F., Elie, C., Nadal, M., Bouthier de la Tour, C., Forterre, P., Duguet, M. (1993). Reverse gyrase: a helicase-like domain and a type I DNA topoisomerase in the same polypeptide. Proc. Natl. Acad. Sci. USA **90**, 4753–4757.
3. Forterre, P., Benachenhou, N., Confalonieri, F., Duguet, M., Elie, C., Labedan, B. (1993). The nature of the last universal ancestor and the root of the tree of life, still open questions. BioSystems **28**, 15–32.
4. Forterre, P., Elie, C. (1993). Chromosome structure, DNA polymerases and topoisomerases in Archaebacteria (Archaea). *In* Kates, M., Kuschner, D. J., Matheson, A. T. (ed.), The Biochemistry of Archaea (Archaebacteria). New Comp. Biochem. **26**, 325–366.
5. Forterre, P. (1995). Thermoreduction, a hypothesis for the origin of procaryotes. Comptes Rendus de l'Academie des Sciences **318**, 415–422.
6. Forterre, P., Confalonieri, F., Charbonnier, F., Duguet, M. (1995). Speculations on the origin of life and thermophily: review of available information on reverse gyrase suggests that hyperthermophilic procaryotes are not so primitive. Orig. Life Evol. Bios. **25**, 235–249.
7. Pace, N. R. (1991). Origin of life-facing up to the physical setting. Cell **65**, 531–533.
8. Stetter, K. O. (1992). Life at the upper temperature border. *In* Trân Thanh Vân, I. & K., Mounolou, J.-C., Schneider, I., Mc Kay, C. (ed.), Frontiers of Life, pp. 195–220. Editions Frontières, Gif sur Yvette, France.
9. Woese, C. R., Kandler, O., Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria and Eucarya. Proc. Natl. Acad. Sci. USA **87**, 4576–4579.

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In the pursuit of hydrocarbons and their biogenetic origin

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Summary

The study on the biosynthesis of hydrocarbons was initiated after the analyses of existing meteorite samples for hydrocarbons showed that the samples were contaminated with terrestrial materials, among which were common bacteria. The question to be answered was the extent to which bacteria and fungi synthesized aliphatic hydrocarbons, which could contaminate and interfere with the analyses of extraterrestrial samples. Twenty to thirty different bacterial species were initially studied for hydrocarbon biosynthesis. Only the micrococci contained significant quantities of hydrocarbons. These bacteria contained a complex mixture of branched, monounsatured isomers that occurred in tetrad arrangements. The different hydrocarbon patterns demonstrated that a very large percent of the strains in the Micrococcaceae were misidentified and the correct lineage could be predicted from the hydrocarbon patterns.

Key words: bacterial hydrocarbons, extraterrestrial samples, *Micrococcus, Pseudomonas*, neutral lipids

Resumen

Los análisis de hidrocarburos de las muestras existentes de meteoritos han demostrado que dichas muestras estaban contaminadas por materiales terrestres, entre ellos bacterias comunes. El problema consiste en determinar si los hidrocarburos alifáticos sintetizados por bacterias y hongos pueden interferir en los análisis de muestras extraterrestres. Con este fin, se estudió la biosíntesis de hidrocarburos de entre 20 y 30 especies bacterianas. Sólo los micrococos contenían una cantidad significativa de hidrocarburos, que consistían en una mezcla compleja de isómeros monoinsaturados ramificados,

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agrupados en tétradas. Se ha demostrado que una parte importante de las cepas de Micrococcaceae habían sido identificadas de manera incorrecta, y que los patrones de hidrocarburos pueden utilizarse para una clasificación correcta.

Introduction

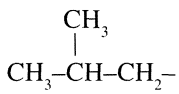
The initial aim of this work was to identify those organic compounds which were characteristic of biotic systems and differentiate them from those produced abiotically. The specific purpose was to establish standards to analyze the samples to be collected in the forthcoming NASA space flights. We chose aliphatic hydrocarbons based on several principles. Pristane (C_{19}) and phytane (C_{20}) were established sediment markers, assumed to derive from the photosynthetic pigment chlorophyll. These hydrocarbons could survive geological conditions and time and they were not considered to be produced abiotically. This study was initiated by analyzing existing meteoritic samples for hydrocarbons. The analyses clearly showed, however, that the samples were contaminated with terrestrial materials, a portion of which consisted of common bacteria (27, 30). The question to be answered was the extent to which bacteria and fungi synthesized aliphatic hydrocarbons which could contaminate and interfere with the analyses of extraterrestrial samples. Only a few studies reported hydrocarbons in bacteria (11, 57), but without conclusive evidence that they were biosynthesized products.

The immediate problem was to establish that microorganisms did synthesize hydrocarbons. Accordingly, analytical methods, such as cultivation, extraction, fractionation and identification procedures were critical steps to assure the authenticity of the results. In addition, all analyses were to be conducted on minute sample sizes, similar to those proposed for extraterrestrial samples, requiring high sensitivity of detection. Thus, analytical methods and tools had to be developed. High resolution capillary columns for gas chromatographic analyses of complex mixtures of non-polar organics were used in the laboratory of J. Oró at the University of Houston. In the early 1960's it was standard procedure to analyze samples eluted from 30 to 100 meters long stainless steel capillary columns operated in gas chromatographs (28, 29). This was years before the now common fused silica capillary columns were developed. The samples eluted from these columns were characterized by the new method of combined gas chromatography–mass spectrometry (GC–MS). Gas chambers attached to vibrating Reed detectors were also constructed at the end of the eluting gases to detect the radioisotopes incorporated into the biochemical markers. This was done to verify them as biosynthesized products. Accordingly, some of the pioneering work in bioanalytical chemistry was conducted in Oró's laboratory.

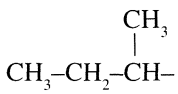
Hydrocarbon composition of bacteria

Twenty to thirty different bacterial species were initially studied for hydrocarbon biosynthesis. The most exciting results proved to be hydrocarbons detected from extrinsic sources, such as hand-creams, vacuum pump oil, laboratory dust and vacuum grease (26, 35). Additional studies were conducted with marine and freshwater organisms, with short chain hydrocarbons being detected in minute quantities (31). We then analyzed the bacterium *Sarcina lutea* ATCC 533. [The genus *Sarcina* was renamed

Micrococcus; see p. 235.] The cellular hydrocarbons (Table 1) consisted of a complex mixture of branched, monounsaturated isomers that occurred in tetrad arrangements (41, 42, 46, 48). The principal clue for the exact configuration of the hydrocarbons resulted from radioisotope incorporation experiments (48), with ^{14}C labeled amino acids and fatty acids revealing the assemblage sequence. It was established that leucine and valine, after deamination and decarboxylation, were the precursors of the iso- configuration, while isoleucine was the precursor of the anteiso- configuration in the synthesis of the methyl branched fatty acids (46, 48).

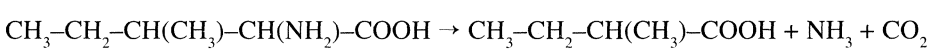


Iso



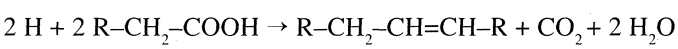
Anteiso

The loss of one $^{14}CO_2$ during the carboxyl end to carboxyl end condensation of two fatty acids, with one carboxyl end reduced to a vinyl unsaturate to form the hydrocarbons, was the key. The identities of the hydrocarbons were then hypothetically constructed by combining the cellular fatty acids. These predicted identities were later confirmed (46). Consequently, the dual end methyl branching arrangement with the iso configuration (iso-iso') occurred at both ends of the even and odd numbered chains; however, the anteiso configuration (anteiso-anteiso) occurred only in the odd numbered chains since only odd numbered carbon fatty acids with the anteiso configuration are possible (42, 46). For example,



isoleucine 2-methyl butyric acid

thus,



Our laboratories (46, 48) and that of Albro and Dittmer (1) and Albro et al.(2) indepently delineated the mechanics of the biochemical pathway at the same time. There was a major discrepancy between the two studies, however, since the hydrocarbons of *S. lutea* analyzed by Albro and Dittmer were in the range from C_{25}–C_{30} while our organism's hydrocarbons were in the range from C_{23}–C_{27} (Table 1). Albro and Dittmer subsequently renumbered their strain to FD 533 since they obtained it from the Fort Dietrick stock collection. Interested in this difference, we analyzed over 100 genera and species in the taxonomic family Micrococcaceae. The hydrocarbons were a major cytoplasmic membrane constituent of micrococci but not staphylococci. In addition, it was discovered that all species of "true" micrococci contained

TABLE 1. Hydrocarbons of *Sarcina lutea* and *Pseudomonas maltophilia*

	S. lutea[a]			*P. maltophilia*[b]
Identity	ATCC 533 Moles %	FD 533 Moles %	Double bond position(s)	Moles %
i, i C23	2.6	–	11:12	–
ai, i C23	1.8	–	11:12	–
ai, ai C23	0.7	–	ND	–
i C23	0.3	–	ND	–
i, i C24	5.5	–	12:12	–
ai, i C24	5.2	–	12:12; 11:13	–
ai, ai C24	–	–	–	–
i C24	1.2	–	12:12; 11:13	–
ai C24	2.1	–	11:13	–
i, i C25	6.0	0.1	12:13	–
ai, i C25	20.8	0.1	12:13	–
ai, ai C25	12.6	0.5	12:13	–
i C25	0.5	0.1	ND	–
i, i C26	2.2	1.5	13:13; 12:14	–
ai, i C26	7.2	1.4	13:13; 12:14	–
ai, ai C26	–	–	–	–
i C26	1.0	0.1	13:13	–
ai C26	4.5	0.2	13:13	–
i, i C27	1.1	0.8	13:14	1.5
ai, i C27	8.1	16.5	13:14; 12:15	–
ai, ai C27	16.6	5.9	13:14; 12:15	0.9
i C27	–	3.5	13:14	1.2
ai C27	–	8.9	13:14	0.9
n C27	–	–	–	0.7
i, i C28	–	2.8	13:15	0.3
ai, i C28	–	5.0	14:14	–
ai, ai C28	–	–	–	–
i C28	–	5.4	13:15; 14:14	4.7
ai C28	–	13.4	13:15; 14:14	5.0
n C28	–	–	–	1.7
i, i C29	–	0.4	14:15	14.2
ai, i C29	–	5.0	14:15	–
ai, ai C29	–	18.4	14:15	4.6
i, ai C29	–	–	–	6.0
i C29	–	2.1	14:15	2.6
ai C29	–	2.8	14:15	2.3
n C29	–	–	–	7.0

Continued on following page

TABLE 1.—*Continued*

	S. lutea[a]			*P. maltophilia*[b]
Identity	ATCC 533 Moles %	FD 533 Moles %	Double bond position(s)	Moles %
i, i C30	–	0.4	ND	4.2
ai, i C30	–	0.4	ND	–
ai, ai C30	–	–	–	–
i C30	–	1.4	15:15	11.1
ai C30	–	3.3	15:15	11.1
i, i C31	–	–	–	1.9
i C31	–	–	–	1.2
ai C31	–	–	–	0.7
n C31	–	–	–	14.9
i C32	–	–	–	0.9

i = iso methyl branch; i, i = iso methyl branches on both ends of the carbon chain; ai = anteiso methyl branch; ai, ai = anteiso branches on both ends of the carbon chain; n = normal; ND = not determined.

[a] Separation on a 99 m × 0.75 mm stainless steel column coated with 3% OV-17.

[b] Gas chromatographic separations on a 62 m × 0.5 mm stainless steel column coated with 10% Apiezon L. More than one value for double bonds denotes isomeric mixtures.

distinct hydrocarbon patterns (15, 24, 47). As a result, we were able to demonstrate that a very large percent of the bacteria in the Micrococcaceae were misidentified (15, 32) and correct lineage could be predicted from the hydrocarbon patterns. The *Sarcina lutea* strains FD 533 and ATCC 533 were renamed *Micrococcus luteus* and *Micrococcus varians*, respectively (15, 24, 32).

Finally, we had proof of specific microbial hydrocarbon patterns. However, shortly after leaving Oró's laboratory, in the laboratory of Morris Kates, at the National Research Council of Canada,we discovered that there was a predominance of isoprenoid hydrocarbons in the neutral lipid extract of *Halobacterium cutirubrum*. We then identified the oxidized and reduced forms of squalene (37, 50).

We then analyzed methanogenic bacteria obtained from Ralph Wolfe at the University of Illinois, starting with *Methanobacterium thermoautotrophicum*. When we extracted the free lipids and saponified them, the glyceride lipids were still intact (49). These lipids were immediately recognized as having the exact same properties as the ether lipids of *Halobacterium cutirubrum* that had been extensively reported by Kates (13). The lipids of the methanogens (Fig. 1), however, were different from the diphytanyl diether glycerols identified by Kates in *Halobacterium* by containing both diethers and tetraethers (23, 44, 45, 50). Additional methanogenic strains studied (10, 22, 44, 45) differed with some containing only the diether structures and others containing both diethers and tetraethers. These tetraether lipids had been previously recognized only in the extreme thermoacidophilic bacteria *Thermoplasma* and *Sulfolobus* and are thought to have been unprecedented in nature (17, 20, 21). Since these initial publications, an outpouring of data from numerous research groups quickly gathered sufficient evidence to propose the identification of a "third form of life" now collectively known as the Archaeobacteria (54, 55, 56). The list of Archaeobacteria now include both autotrophs and heterotrophs

as well as mesophiles, thermophiles, halophiles, acidophiles and alkaliphiles and combinations thereof. The variations in the chemical nature of the polar lipid fractions also increased (Fig. 1) with the discovery of macrocyclic ethers (4), cyclopentyl tetraethers (6), iso- and anteiso-heptadecyl glycerol diethers (19), and 3'-hydroxyphytanyl glycerol diethers (8). These data as well as the descriptions of the complete lipid structures have been described in numerous reviews, some of which are listed here (5, 7, 12, 13, 14, 16, 18, 22).

Neutral lipids of methanogens, representing from 2 to 40% of the total lipids, are comprised predominantly of isopranyl and isoprenyl hydrocarbons (10, 19, 22, 45, 50). The distribution of the hydrocarbons found were in the range from C_{16} to C_{30} with no predominance of even/odd numbered carbons (Fig. 2). The structure of some of the unconventional chain lengths (all of them except C_{20} and C_{30}) are obviously synthesized by a pathway different from those of classical isopranes. The diversity of these types of hydrocarbons in other Archaeobacteria were even greater with additional isomeric forms (22). Other noted neutral lipids identified included nathoquinones (43) and alkyl benzenes (22). Although the neutral lipids of the Archaeobacteria are complex and relatively distinct, they are unlike those of the micrococci discussed earlier and do not differentiate the genera or species.

At this point in time, it appeared that the discovery of other bacteria that synthesized hydrocarbons was unlikely (3, 34, 38, 39, 40). Along with the two hydrocarbon studies, however, was a project on

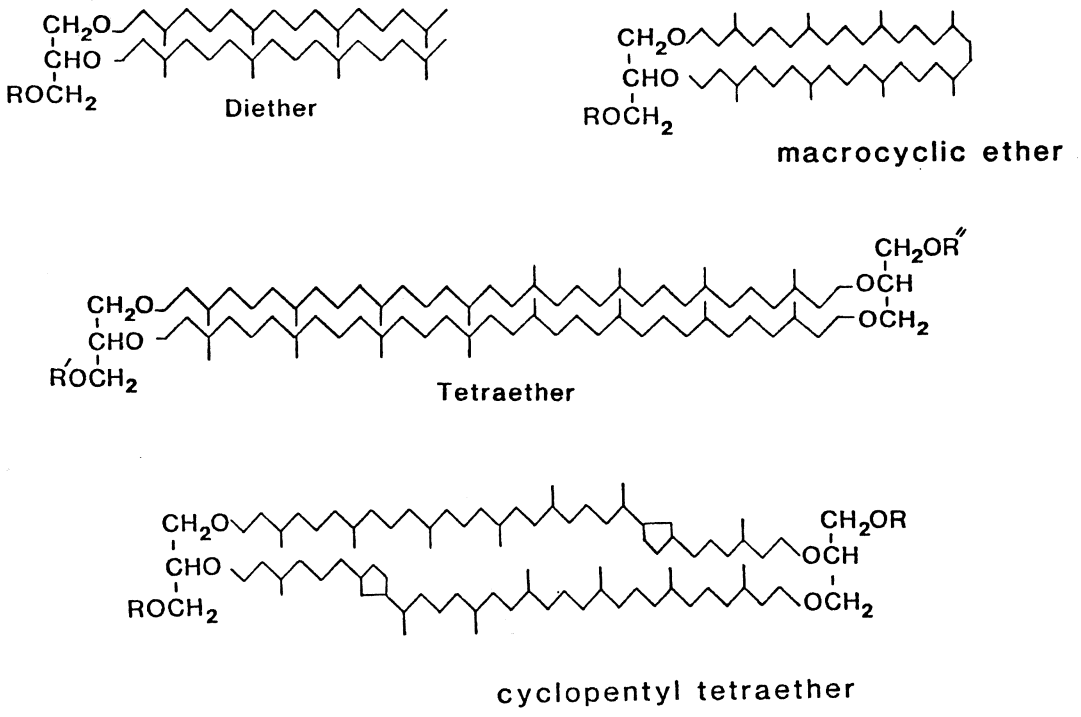


FIG. 1. Lipids of methanogens.

typing the endotoxins of *Yersinia pestis*, the causative agent of bubonic plague. As a routine procedure with all isolates the cells were analyzed for hydrocarbons. There were none (36). But, in the course of studying the pathogenicity of the plague organisms, we found that the majority of the isolates were contaminated with a bacterium identified as *Pseudomonas maltophilia*. This bacterium had hydrocarbons. Fifteen referenced strains of *P. maltophilia* were analyzed, each of them demonstrating similar hydrocarbon patterns (49). The hydrocarbons were unique in that they had polyunsaturated chains (33, 49), a feature not characteristic of bacteria with the exception of cyanobacteria. In the photosynthetic apparati of cyanobacteria, the lipid components contain 18:1, 18:2 and 18:3 fatty acids. The identity of the hydrocarbons are given in Tables 1 and 2. The hydrocarbon configurations were similar to those of the micrococci with iso- and anteiso- branches on one or both ends of the chains. The interesting feature of these pseudomonads is the way the hydrocarbons are synthesized. The relative distribution and position of the double bonds could be manipulated, in part, by the nature of the fermentation acids present in the metabolic pool. Extrinsic supplies of metabolic intermediates (33) demonstrated that the bacterium controlled its fermentation pool by condensing the acids into hydrocarbons with the double bond occurring at the site of the condensation. Thus, the two and three double bonds were from the condensation of two or three fermentation acids, respectively. This mechanism is similar yet different from the pathway in micrococci, described earlier, which synthesized the hydrocarbons from longer chain fatty acids that were equivalent to those found in the membrane lipids (1, 46, 48).

In conclusion, only a few bacterial species synthesize significant quantities (relative to the total cellular lipids) of acyclic hydrocarbons. The bacterial hydrocarbons are a specific class of compounds that are terminal products, apparently synthesized as part of the metabolic regulatory process.

The significance of this study, however, has provided a number of major contributions as delineated in the report. The primary objective of using these data for establishing guidelines for determining if life existed elsewhere in the universe is now just an amusing part of this report. The use of these data as biomarkers, however, has been well utilized in the fields of taxonomy (32) and biomass detection and characterization (9, 25, 51, 52, 53). The lipids are indicators of biological activities in bioremediation

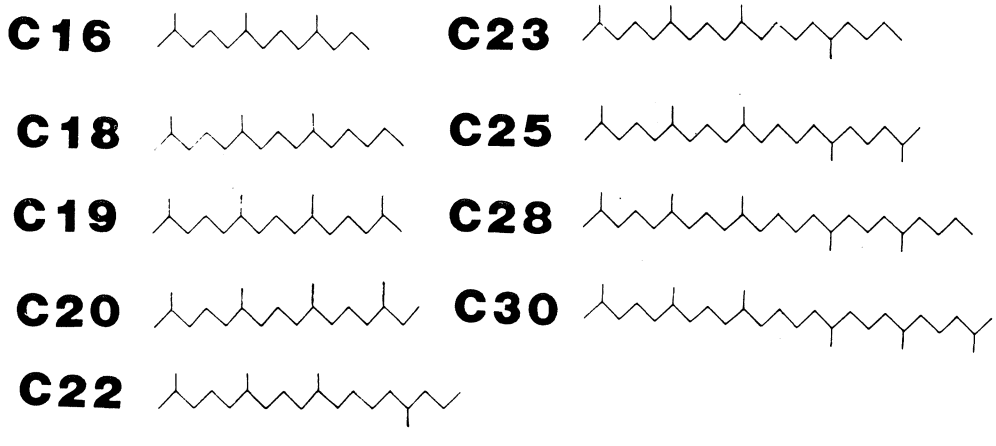


FIG. 2. Chain length distribution of neutral lipids of methanogens.

TABLE 2. Alkene composition of hydrocarbons of *Pseudomonas maltophilia* and relative position of double bonds in C 29 and C 30 hydrocarbons*

Alkenes	Moles %	Double bond	Configuration
C 26:2	0.4		
C 26:1	0.5		
C 27:3	0.6		
C 27:2	4.6		
C 27:1	3.1		
C 28.3	1.3		
C 28:2	5.0		
C 28:1	3.1		
C 29:3	10.0	(Δ8, 14, 21)	C_7H_{15}–CH=CH–C_4H_8–CH=CH–C_5H_{10}–CH=CH–C_7H_{15}
C 29:2	24.1	(Δ8, 14)	C_7H_{15}–CH=CH–CH_8–CH=CH–$C_{14}H_{29}$
C 29:1	6.2	(Δ14)	$C_{13}H_{27}$–CH=CH–$C_{14}H_{29}$
C 30:3	8.1	(Δ8, 14, 21)	C_7H_{15}–CH=CH–C_4H_8–CH=CH–C_5H_{10}–CH=CH–C_8H_{17}
C 30:2	9.9	(Δ9, 16)	$C_{13}H_{27}$–CH=CH–C_5H_{10}–CH=CH–C_8H_{17}
C 30:1	2.0		
C 31:3	16.6		
C 31:2	4.5		

* See Table 1 for description of branching configurations. Separation on a 30 m × 0.02 mm OV-351 fused silica capillary column with 0.25 μm film. The identities were determined by GC–MS of TMS-derivatives of the oxygenated alkenes.

studies, biofouling and chemotaxonomy. In our laboratory, we are currently using the lipids to map the succession of changes in the consortium of microorganisms that occur in experimental solid state fermentation systems.

References

1. Albro, P. W., Dittmer, J. C. (1970). Bacterial hydrocarbons: occurrence, structure and metabolism. Lipids **5**, 320–325.
2. Albro, P. W., Meeham, T. D., Dittmer, J. C. (1970). Intermediate steps in the incorporation of fatty acids into long chain, non-isoprenoid hydrocarbons by lysates of *Sarcina lutea*. Biochemistry **9**, 1893–1898.
3. Ben-Amotz, A., Tornabene, T. G., Thomas, W. H. (1985). Chemical profiles of selected species of microalgae with the emphasis on lipids. J. Phycol. **21**, 72–81.
4. Comita, P. B., Gagosian, R. B., Pang, H., Costello, C. E. (1984). Structural elucidation of a unique macrocyclic membrane lipid from a new, extremely thermophilic, deep sea hydrothermal vent Archaebacterium, *Methanococcus jannaschii*. J. Biol. Chem. **259**, 15234–15241.
5. de Rosa, M., de Rosa, S., Gambacorta, A., Minale, L., Bu'lock, J. D. (1977). Chemical structure of the ether lipids of thermophilic acidophilic bacteria of the *caldariella* group. Phytochemistry **16**, 1961–1965.
6. de Rosa, M., de Rosa, S., Gambacorta, A., Bu'lock, J. D. (1980). Structure of calditol, a new branched chain nonital, and of the derived tetraether lipids in thermoacidophile Archaebacteria of the *caldariella* group. Phytochemistry **19**, 249–254.

7. de Rosa, M., Gambacorta, A., Nicolaus, B., Sodano S., Bu'lock, J. D. (1980). Structural regularities in tetraether lipids of *caldariella* and their biosynthetic and phyletic implications. Phytochemistry **19**, 388–836.
8. Ferrante, G., Ekiel, I., Patel, G. B., Sprott, G. D. (1988). A novel core lipid isolated from aceticlastic methanogen, *Methanotrix concilii*. Biochim. Biophys. Acta **963**, 173–182.
9. Geesey, G. G., White, D. C. (1990). Determination of bacterial growth and activity at solid-liquid interfaces. Annu. Rev. Microbiol. **44**, 579–602.
10. Holzer, G., Oró, J., Tornabene, T. G. (1979). Gas chromatographic–mass spectrometric analyses of neutral lipids from methanogenic and thermoacidophilic bacteria. J. Chromatogr. **186**, 795–809.
11. Janowski, G. J., ZoBell, C. E. (1944). Hydrocarbon production by sulfate reducing bacteria. J. Bacteriol. **47**, 447.
12. Jones, W. J., Nagle, D. P., Whitman., W. B. (1987). Methanogens and the diversity of Archaebacteria. Microbiol. Rev. **51**, 135–177.
13. Kates, M. (1978). The phytanyl ether-linked polar lipids and isoprenoid neutral lipids of extremely halophilic bacterial. Prog. Chem. Fats & Other Lipids **15**, 301–342.
14. Kates, M. (1990). Glyco-, phosphoglyco- and sulfoglycoglycerolipids of bacteria. *In* Kates, M. (ed.), Glycolipids, Phosphoglycolipid and Sulfoglycolipids, vol. 6, pp. 1–122. Handbook of Lipid Research, Plenum Press, New York, NY.
15. Kloos, W. E., Tornabene, T. G., Schleifer, K. H. (1974). Isolation and characterization of micrococci from human skin. Int. J. Syst. Bacteriol. **24**, 79–101.
16. Koga, Y., Nishihara, M., Morii, H., Akagawa-Matsushita, M. (1993). Ether polar lipids of methanogenic bacteria: structures, comparative aspects, and biosynthesis. Microbiol. Rev. **57**, 164–182.
17. Langworthy, T. A. (1977). Long chain diglycerol tetraethers from *Thermoplasma acidophilum*. Biochim. Biophys. Acta **487**, 37–50.
18. Langworthy, T. A. (1985). Lipids of Archaebacteria. *In* Woese, R. C., Wolfe, R. S. (ed.), The Bacteria, vol. 8, pp. 459–497. Academic Press, Orlando, FL.
19. Langworthy, T. A., Holzer, G., Zeikus, J. G., Tornabene, T. G. (1983). Iso and anteiso- branched glycerol diethers of the thermophilic anaerobe *Thermodesulfotobacterium commune*. Syst. Appl. Microbiol. **4**, 1–17.
20. Langworthy, T. A., Smith, P. E., Mayberry, W. R. (1972). Lipids of *Thermoplasma acidophilum*. J. Bacteriol. **112**, 1193–1200.
21. Langworthy, T. A., Smith, P. E,. Mayberry, W. R. (1974). Long chain diether and polyol dialkyl triether-lipid of *Sulfolobus acidocaldarius*. J. Bacteriol. **119**, 106–116.
22. Langworthy, T. A., Tornabene, T. G., Holzer, G. (1982). Lipids of Archaebacteria. Zbl. Bakt. Hyg. **3**, 228–244.
23. Makula, R. A., Singer, M. E. (1978). Ether containing lipids of methanogenic bacteria. Biochem. Biophys. Res. Commun. **82**, 716–722.
24. Morrison, S. J., Tornabene, T. G., Kloos, W. E. (1971). Neutral lipids in the study of relationship of members of the family *Micrococcaceae*. J. Bacteriol. **108**, 353–358.
25. Nichols, P. D., Mancuso, C. A., White, D. C. (1987). Assessment of methanotrophic and methanogenic biomass and community structure from environmental samples using signature phospholipids. Org. Geochem. **11**, 451–461.
26. Nooner, D. W. (1966). Alkanes in meteorites and terrestrial samples. Ph. D. Thesis, University of Houston, Houston, TX.
27. Oró, J. (1965). Comparative study of the hydrocarbons in Orgueil, Murray and Mokoia meteorites. 27th Annual Meeting of Meteoritical Soc. Arizona State University, Tempe, AZ.
28. Oró, J., Gelpí, E. (1968). Gas chromatography–mass spectrometry studies on the isoprenoid and other isomeric alkanes in meteorites. Int. Symp. on Meteorite Research. Vienna, Austria.
29. Oró, J., Nooner, D. W., Zlatkis, A. S., Wikström, A. (1966). Paraffinic hydrocarbons in the Orgueil, Murray, Mokoia and other meteorites. *In* Life Sciences and Space Research, vol. 4, pp. 63–100. Spartan Books, Washington, DC.
30. Oró, J., Tornabene, T. G. (1965). Bacterial contamination of some carbonaceous meteorites. Science **150**, 1046–1048.
31. Oró, J., Tornabene, T. G., Nooner, D. W., Gelpí, E. (1967). Aliphatic hydrocarbons and fatty acids of some marine and freshwater organisms. J. Bacteriol. **93**, 1811–1818.

32. Schleifer, K. H. (1989). Family I. Micrococcaceae. *In* Staley, J. T., Bryant, M. P., Pfennig, N., Holt, J. G. (ed.), Bergey's Manual of Systematic Bacteriology, vol. 2, pp. 1003–1007. Williams & Wilkins, Baltimore, MD.
33. Suen, Y., Holzer, G., Hubbard, J. S., Tornabene, T. G. (1988). Biosynthesis of acyclic methyl branched polyunsaturated hydrocarbons in *Pseudomonas maltophilia*. J. Indust. Microbiol. **2**, 337–348.
34. Suen, Y., Hubbard, J. S., Holzer, G., Tornabene, T. G. (1987). Total lipid production of the green alga *Nannochloropsis* sp. QII, under different nitrogen regimes. J. Phycol. **23**, 289–296.
35. Tornabene, T. G. (1967). Distribution and biosynthesis of microbial hydrocarbons. Ph. D. Thesis, University of Houston, Houston, TX.
36. Tornabene, T. G. (1973). Lipid composition of selected strains of *Yersinia pestis* and *Yersinia pseudotuberculosis*. Biochim. Biophys. Acta. **306**, 173–185.
37. Tornabene, T. G. (1978). Non-aerated cultivation of *Halobacterium cutirubrum* and its effect in cellular squalenes. J. Mol. Evol. **11**, 253–257.
38 Tornabene, T. G. (1981). Formation of hydrocarbons by bacteria and algae. *In* Hollaender, A., Rabson, R., Rogers, P., Pietro, J., Valentine, R., Wolfe, R. (ed.), Trends in the Biology and Fermentation for Fuels and Chemicals, pp. 421–438. Plenum Press, New York, NY.
39. Tornabene, T. G. (1982). Microorganisms as hydrocarbon producers. Experientia **38**, 43–46.
40. Tornabene, T. G. (1985). Lipid analysis and the relationship to chemotaxonomy. Meth. Microbiol. **18**, 209–234.
41. Tornabene, T. G., Bennett, E. O., Oró, J. (1967). Fatty acids and hydrocarbon composition of *Sarcina lutea* grown in three different media. J. Bacteriol. **94**, 344–348.
42. Tornabene, T. G., Gelpi, E., Oró, J. (1967). Identification of fatty acids and hydrocarbons in *Sarcina lutea* by gas chromatography and combined gas chromatography–mass spectrometry. J. Bacteriol. **94**, 333–343.
43. Tornabene, T. G., Kates, M., Gelpí, E., Oró, J. (1969). Occurrence of squalene, di and tetrahydrosqualene and vitamin MK8 in an extremely halophilic bacterium *Halobacterium cutirubrum*. J. Lipid Res. **10**, 294–303.
44. Tornabene, T. G., Langworthy, T. A. (1978). Diphytanyl and dibiphytanyl glycerol ether lipids of methanogenic Archaebacteria. Science **203**, 51–53.
45. Tornabene, T. G., Langworthy, T. A., Holzer, G., Oró. J. (1979). Squalenes, phytanes, and other isoprenoids as major neutral lipids of methanogenic and thermoacidophilic Archaebacteria. J. Mol. Evol. **13**, 73–83.
46. Tornabene, T. G., Markey, S. P. (1971). Characterization of branched mononunsaturated hydrocarbons of *Sarcina lutea* and *Sarcina flava*. Lipids **6**, 190–195.
47. Tornabene, T. G., Morrison, S. J., Kloos, W. E. (1970). Aliphatic hydrocarbon contents of various members of the family *Micrococcaceae*. Lipids **5**, 929–937.
48. Tornabene, T. G., Oró, J. (1967). ^{14}C incorporation into fatty acids and hydrocarbons of *Sarcina lutea*. J. Bacteriol. **94**, 349–358.
49. Tornabene, T. G., Peterson, S. L. (1978). *Pseudomonas maltophilia*: identification of the hydrocarbons, glycerides and glycolipoproteins of cellular lipids. Can. J. Microbiol. **24**, 525–532.
50. Tornabene, T. G., Wolfe, R. S., Balch, W. E., Holzer, G., Fox, G. E., Oró, J. (1978). Phytanyl-glycerol ethers and squalenes in Archaebacterium *Methanobacterium thermoautotrophicum*. J. Mol. Evol. **11**, 259–266.
51. Tunlid, A., White, D. C. (1990). Use of lipid biomarkers in environmental samples. *In* Fox, A., Morgan, S. L., Larsson, L., Odham, G. (ed.), Analytical Microbiology Methods, pp. 259–274. Plenum Press, New York, NY.
52. Vella, A. J., Holzer, G. (1990). Ether derived alkanes from sedimentary organic matter. Org. Biochem. **15**, 209–214.
53. Vella, A. J., Holzer, G. (1992). Distribution of isoprenoid hydrocarbons and alkyl benzenes in immature sediments. Evidence for direct inheritance from bacterial/algal sources. Org. Geo. Chem. **18**, 203–210.
54. Woese, C. R. (1981). Archaebacteria. Sci. Amer. **244**, 98–122.
55. Woese, C. R., Kandler, O., Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archae, Bacteria, Eucarya. Proc. Natl. Acad. Sci. USA **87**, 4576–4579.
56. Woese, C. R., Magrum, L. J., Fox, G. E. (1978). Archaebacterium. J. Mol. Evol. **11**, 245–252.
57. ZoBell, C. E. (1945). The role of bacteria in the formation of petroleum hydrocarbons. Science **109**, 364–368.

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A new theory on the origin and evolution of the citric acid cycle

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Summary

Sunlight or UV photolysis of certain Krebs cycle intermediates stimulates reactions to occur which are analogous to metabolic steps of modern living cells. For example, 0.1 M aqueous oxalacetic acid yields citric acid upon exposure to sunlight in a quartz tube. Results of this nature have led us to the following hypothesis: the origin of the Krebs cycle reactions lies in aqueous photochemical reactions which occurred on the primitive Earth before and during the evolution of the first cells. These photochemical reactions were "recruited" by evolving cells for their own use. That is, primitive polypeptide macromolecules may have provided the crude catalysis needed for these reactions, along with ultraviolet light. As the cells evolved, the requirement for light was eliminated and the mechanisms changed with time from 1 electron radical reactions to the 2 electron ionic mechanisms seen for the most part in the aerobic cellular reactions of the modern Krebs cycle. A survey of the literature has revealed additional support for this hypothesis. Briefly: (i) succinic acid and its photolysis products are components of the Murchison meteorite, (ii) light-dependent Krebs cycle reactions occur in green anoxygenic bacteria, (iii) 2-keto acid oxidations in archaeobacteria occur by a radical mechanism, different (more primitive) from those of modern cells, (iv) flavin-containing microspheres catalyze fundamental metabolic reactions in the presence of light. Collectively, the above results suggest a step-wise scenario for the origin and evolution of the Krebs cycle.

Key words: sunlight/UV photolysis, Krebs cycle compounds, origins of metabolism, pyruvate, coenzymes

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Resumen

La fotólisis de determinados compuestos intermediarios del ciclo de Krebs mediante luz solar o radiación ultravioleta estimula reacciones que son análogas a algunos pasos metabólicos de las células vivas actuales. Por ejemplo, una disolución 0,1 M de ácido oxalacético produce ácido cítrico cuando se expone a la luz del Sol en un tubo de cuarzo. Resultados de este tipo nos han llevado a la siguiente hipótesis: el origen de las reacciones del ciclo de Krebs se encuentra en las reacciones fotoquímicas en medio acuoso que tuvieron lugar en la Tierra primitiva, antes y durante la evolución de las primeras células. Estas reacciones fotoquímicas fueron "reclutadas" por las células para su beneficio propio. Es decir, las macromoléculas polipeptídicas primitivas aportaron catálisis rudimentarias a estas reacciones, junto con la ayuda de la luz ultravioleta. Mientras las células evolucionaban, se perdió la necesidad de la luz y los mecanismos cambiaron, con el tiempo, de reacciones con radicales y 1 electrón de transferencia a mecanismos iónicos con 2 electrones, que son los más frecuentes en las reacciones celulares aeróbicas del ciclo de Krebs moderno. Un estudio de la bibliografía ha aportado una ayuda adicional a esta hipótesis. Brevemente: (i) el ácido succínico y los productos de su fotólisis son componentes del meteorito de Murchison, (ii) las bacterias anoxigénicas verdes tienen reacciones del ciclo de Krebs que dependen de la luz, (iii) las oxidaciones de 2-ceto ácido de las arqueobacterias se producen por un mecanismo de radical, diferente (más primitivo) del de las células modernas, (iv) las microesferas que contienen flavina catalizan reacciones metabólicas fundamentales en presencia de luz. En definitiva, los resultados anteriores sugieren un desarrollo escalonado del origen y la evolución del ciclo de Krebs.

Introduction

The Krebs citric acid cycle (CAC) is the central pathway of energy metabolism in modern aerobic cells. A growing body of knowledge concerning the evolution of this sequence has provided keen insight into the rational structure and design of life processes. Particularly, Baldwin and Krebs (1) described the higher efficiency of a *cyclic* oxidation of acetate, as opposed to a linear one. Gest (6) presented a specific scenario for the evolution of the modern CAC based on the existence of a reductive pathway in primitive microorganisms. The subject of CAC evolution has also been reviewed by Weitzman (23).

Despite this serious attention to the *evolution* of the Krebs cycle in living organisms, very little research has been directed toward its *origin*, i.e. its roots in prebiotic chemistry. Negron-Mendoza and Ponnamperuma (13, 14) have used gamma irradiation to demonstrate the production and interconversion of CAC compounds, thereby providing valuable evidence for the probable occurrence of these intermediates on the primitive Earth. But how did the reactions of the CAC arise? Were primitive polypeptides required to initiate the early stages? What is the evolutionary *origin* of the Krebs citric acid cycle? The purpose of this paper is to begin to answer these questions and to develop an hypothesis (based upon experimental chemistry) that the roots of the CAC lie in prebiotic and early biotic organic photochemistry.

Photochemical results

Table 1 summarizes the results which we have obtained on the sunlight and UV photolysis of CAC intermediates (18, 19, 21). Certain conversions are especially intriguing because they seem to mimic analogous reactions of the Krebs cycle. For example: 2-ketoglutaric acid to succinic acid; oxalacetic to citric acid; fumaric to/from malic and succinic acids; malic to fumaric and succinic acids. The efficient sunlight photolysis of the amino acid glutamic acid to succinic acid, probably via 2-ketoglutaric acid (21), is unusually interesting since these steps mimic the metabolic degradation of glutamic acid in cells.

TABLE 1. Sunlight and UV photolysis of citric acid cycle intermediates[a]

Starting acid	Conditions[b]	Mass recovery (%)	Major products[c]
2-ketoglutaric	0.1 M, Sun,10 wk	–	succinic acid higher molecular weight products
oxalacetic	0.1 M, Sun,10 wk	50	citric acid malonic pyruvic 4-ketopentanoic 4-ketopent-2-enoic acetaldehyde succinic
succinic	Hg lamp, 14 h	26	adipic 3-methylglutaric maleic (fumaric) 2-methylglutaric 2-methylsuccinaldehydic
fumaric	Hg lamp, 14 h	61	malic succinic
malic	Hg lamp, 14 h	18	maleic (fumaric) succinic
citric	Hg lamp, 14 h	39	2-methyl-2-hydroxysuccinic 3-hydroxyglutaric tricarballylic malic succinic
glutamic	0.1 M, Sun, 7 wk pH = 7.0	–	succinic[d]

[a] Identifications were made by GC–MS of derived methyl esters. In most cases, authentic standards were available for direct comparison. When standards were not available, the MS of the unknown was compared to that of published spectra. Dark controls were run in all cases.

[b] Distilled water solutions.

[c] Listed in descending order of abundance.

[d] The calculated yield of succinic acid was variable but reached 43% conversion in one experiment.

Furthermore, we have reported that analogous reactions attempted with *heat* as the energy source do not give meaningful results. That is, it is UV light but not heat which effects the Krebs cycle-like reactions (15).

We propose that aqueous photochemical reactions of CAC compounds were taking place on the primitive Earth, where UV radiation was likely to be a major source of energy for prebiotic chemistry in aqueous environments (5). Support for this notion comes from a surprising area of science. The photolysis products from succinic acid (Table 1) are all components, along with succinic acid itself, of the Murchison carbonaceous meteorite (17). This co-occurrence of succinic acid and its photolysis products in the meteorite suggests (i) that the photolysis of succinic acid did occur in outer space and on the primitive Earth, (ii) that succinic acid may have had an important role in chemical evolution, and (iii) that the UV photolysis of CAC compounds may have occurred on the primordial Earth as proposed (20).

The mechanisms for the reactions of Table 1 are not known with certainty. However, it is clear from the nature of the products and the character of photochemical conversions that free radical intermediates are involved. With that in mind, the mechanisms for the photo-reactions (1 electron transfers) may be analogous to the corresponding mechanisms for the enzymatic reactions in a cell (generally 2 electron transfers). This concept is ingrained in the hypothesis of this paper (*vide infra*) and is illustrated in Fig. 1 for citric acid formation by photochemistry and by the citrate synthetase reaction. The aldol-like addition of a carbon radical to a carbonyl group (Fig. 1) is now a well-recognized event (22). Thus, not only are the observed photo-reactions mimics of cellular CAC reactions, but there also appears to be a relationship (perhaps distant) between their step-wise mechanisms (1 electron vs. 2 electron transfers).

The stage is now set for a statement of the hypothesis.

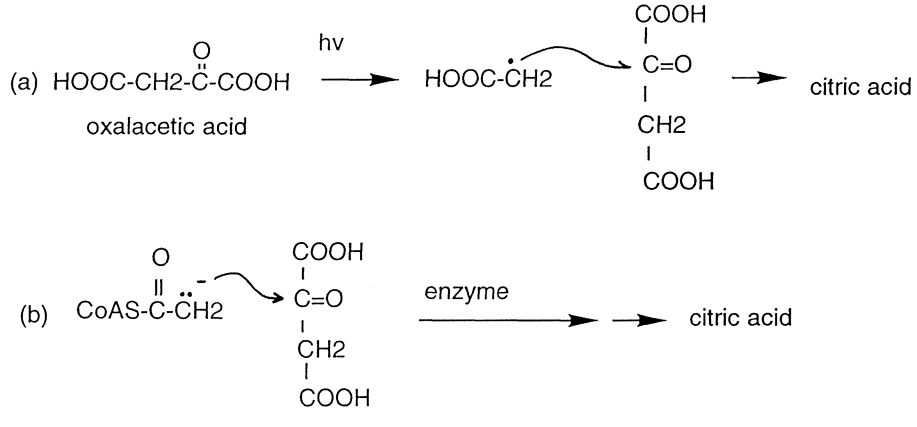

FIG. 1. A comparison of reaction mechanisms. (a) Photochemical formation of citric acid via acetic acid radical (1 electron transfer); (b) enzymatic citrate synthetase reaction (2 electron transfer).

The hypothesis

The origin of the Krebs cycle reactions lies in aqueous photochemical reactions which occurred on the primitive Earth before and during the evolution of the first cells. These photochemical reactions (ancestors of the modern Krebs cycle) were "recruited" by evolving cells for their own use. That is, primitive polypeptide macromolecules may have provided crude catalysis for these reactions, along with the need for ultraviolet light. As the cells evolved, the requirement for light was eliminated and the mechanisms changed with time from 1 electron radical reactions to 2 electron ionic mechanisms seen for the most part in the aerobic reactions of the modern Krebs cycle.

The notion of an important role for UV radiation in prebiotic and early biological chemistry has been proposed by Krasnovsky (10) and discussed by Kritsky (11). According to these concepts, a transition occurred *from* utilization of UV light for fundamental chemistry in primordial cells, with primitive coenzymes (like flavins) as light absorbers, *to* more modern photosynthetic systems where light is not required in substrate metabolism. This general proposal is consistent and supportive of our more specific hypothesis. In fact, as will be seen in the next section, some living microorganisms do use coenzymes as light absorbers in non-photosynthetic processes. In relationship to our hypothesis, we view such a role of light as a retained intermediate stage in an evolutionary change from a light-dependent to a light-independent metabolism.

Additional support for the hypothesis

The photochemical experiments summarized in Table 1 by themselves provide a first line of evidence in support of the hypothesis. That is, if one assumes that these results relate to the origin of life, then some statement akin to the hypothesis is implied. However, additional evidence can be found in the literature of microbiology. Stated in another way, unusual pathways exist in the metabolism of primitive cells which can be explained by the hypothesis of this paper.

(i) The conversion of pyruvate into acetoin is both an in vitro photochemical reaction (12) and an enzymatic step in the metabolism of *Pyrococcus furiosus* (Archaeobacteria) (16). The production of acetoin is not a common metabolic fate of pyruvate, nor is it a thermal product of pyruvate.

$$2\ CH_3COCOOH \rightarrow CH_3{-}\overset{\overset{O}{\|}}{C}{-}\overset{\overset{OH}{|}}{\underset{\underset{H}{|}}{C}}{-}CH_3 + 2\ CO_2$$

According to the hypothesis, the prebiotic photo-conversion of pyruvate to acetoin was recruited by early cells and a polypeptide catalyst evolved such that the requirement of light was eventually removed, and the mechanism changed from the photochemical 1 electron transfers to the 2 electron transfers requiring the action of a coenzyme (thiamin in this case).

(ii) In the green alga *Chlorella* where a *light-activated* amino acid oxidase (amino acid → 2-keto acid) involves light absorption by a flavin cofactor (7). This process is analogous to the photochemical

deamination of glutamate (glutamate → 2-keto acid → succinate) reported in Table 1. The requirement of light in this amino acid oxidase reaction, and the light absorption by the cofactor, may be an example of "paleometabolism", an intermediate stage in the eventual elimination of light-dependency from this enzymatic step.

(iii) A light-dependent citric acid cycle has been observed in *Chlamydomonas*, another green alga (24). The process involves light-absorption by PS-I, followed by PS-I (oxid) acting as an acceptor of electrons from NAD/$FADH_2$ generated in the Krebs cycle. This scheme does not exist in higher plants. Our hypothesis states that the reactions of the CAC were, at the beginning, light-dependent at the substrate-level. The green algae have evolved beyond this primitive state but the requirement of light for the reactions has not been eliminated as it has in more highly evolved cells.

(iv) A photo-oxidation of succinic acid occurs in the photosynthetic bacterium *Rhodospirillum rubrum* in the reation: succinate +NAD → fumarate+NADH (4). Analogously, Table 1 records our non-enzymatic succinic to fumaric acid photo-conversion.

(v) Pyruvate and 2-ketoglutarate oxidations in archaeobacteria use different enzymes and mechanisms than the same oxidations in higher organisms (2, 3). Ancestral type 2-keto acid ferredoxin oxidoreductases catalyze the reactions $RCOCOO^- \rightarrow RCOSCoA + CO_2$ with 1 electron transfers and radical intermediates. We have observed (Table 1) that 2-keto acids are photo-oxidized by sunlight with free radical intermediates. That the 2-keto acids in archaeobacteria are uniquely oxidized also through radical intermediates supports our hypothesis of a 1 electron → 2 electron mechanism change during the evolution of the CAC from an early dependency on UV light.

(vi) Finally, in a chemical evolution experiment, flavin-containing proteinoid microspheres catalyze certain fundamental metabolic-like reactions in the presence of light (9). If one accepts that microspheres are relevant to the origin of life, then this experiment can be interpreted to mean that UV light-dependent, prebiotic, metabolism-like chemistry was also relevant to the origin of fundamental pathways like the CAC.

Scenario for the origin and evolution of the CAC

The hypothesis of this paper, now buttressed by observed photochemical reactions, the metabolism of primitive microorganisms, and a chemical evolution experiment with microspheres, can be re-written in scenario form.

(i) Non-enzymatic CAC-like reactions were occurring photochemically on the primordial Earth.

(ii) The first cells made use of these light-dependent reactions with the assistance of polypeptide catalysts. Here, there was a direct interaction of UV radiation with substrate molecules.

(iii) With the appearance of photosynthetic organisms, oxygen accumulation, and the evolution of more efficient protein catalysts, cells evolved which required light only for certain steps, with coenzymes acting as light-absorbers.

(iv) Finally, the former requirement for light was eliminated, giving rise to a light-independent ancestral cell.

One can imagine powerful selective forces favouring these evolutionary changes. With the accumulation of oxygen in the atmosphere and the resultant decrease in the flux of UV radiation on the planet surface, cells had to develop chromophores (coenzymes) more efficient as light-absorbers than the substrate molecules themselves. In addition, free radicals react rapidly with oxygen, so the reaction mechanisms had to change from 1 electron to 2 electron transfers. The elimination of light-dependency entirely allowed cells to radiate into dark environments and to form multicellular structures.

Conclusion

A good theory explains observations and provides a framework for interpreting a body of knowledge. In this paper we have made a case that our hypothesis (and scenario) accomplishes this goal. However, a good theory also makes predictions. We predict that an example will be found in a primitive microorganism where pyruvate and/or 2-ketoglutarate (light-sensitive acids) are directly photolyzed, perhaps enzyme-assisted, in fundamental steps of metabolism. Such examples might exist in nature, as yet undetected. Indeed, the molecular mechanisms of light-dependent processes of a non-photosynthetic nature have not been thoroughly investigated (11).

In conclusion, we are gratified to note that organic photochemistry involving keto-acids is taking place today in sea water (8). It is taking place today and it very likely took place before and during the origin of life.

References

1. Baldwin, J. E., Krebs, H. (1981). The evolution of metabolic cycles. Nature **291**, 381–382.
2. Blamey, J. M., Adams, M. W. (1994). Characterization of an ancestral type of pyruvate ferredoxin oxidoreductase from the hyperthermophilic bacterium *Thermotoga maritima*. Biochemistry **33**, 1000–1007.
3. Danson, M. J. (1989). Central metabolism of the Archaebacteria. Can. J. Microbiol. **35**, 58–64.
4. Evans, M. C. W. (1965). The photo-oxidation of succinate by chromatophores of *Rhodospirillum rubrum*. Biochem. J. **95**, 661–668.
5. Fox, S. W., Dose, K. (1977). Molecular evolution and the origin of life (revised ed.). Marcel Dekker, New York, NY.
6. Gest, H. (1981). Evolution of the citric acid cycle and respiratory energy conversion in prokaryotes. FEMS Microbiol. Lett. **12**, 209–215.
7. Kamiya, A., Kowallik, W. (1991). Influences of blue light on uptake and consumption of amino acids in a colorless mutant of *Chlorella*. J. Plant Physiol. **138**, 279–284.
8. Kieber, D. J., Mopper, K. (1987). Photochemical formation of glyoxylic and pyruvic acids in seawater. Mar. Chem. **21**, 135–149.
9. Kolesnikov, M. P. (1992). Flavin-dependent processes in model prebiological systems. Izv. Akad. Nauk. Ser. Biol. **6**, 844–853.

10. Krasnovsky, A. A. (1974). Chemical evolution of photosynthesis: models and hypotheses. *In* Dose, K. H., Fox, S. W., Deborin, G. A., Paulovskaya, T. E. (ed.), The Origin of Life and Evolutionary Biochemistry, pp. 233–244. Plenum Press, New York, NY.
11. Kritsky, M. S. (1974). The light-dependent processes in fungi: a possible approach to some problems of photobiological evolution. *In* Dose, K. H., Fox, S. W., Deborin, G. A., Paulovskaya, T. E. (ed.), The Origin of Life and Evolutionary Biochemistry, pp. 263–269. Plenum Press, New York, NY.
12. Leermakers, P. A., Vesley, G. F. (1963). The photochemistry of 2-keto acids and esters. J. Am. Chem. Soc. **85**, 3776–3779.
13. Negron-Mendoza, A., Ponnamperuma, C. (1976). Formation of biologically relevant carboxylic acids during the gamma irradiation of acetic acid. Origin of Life **7**, 191–196.
14. Negron-Mendoza, A., Ponnamperuma, C. (1978). Interconversion of biologically important carboxylic acids by radiation. Origin of Life **9**, 101–104.
15. Osborne, C. B., Waddell, T. G. (1982). Experimental investigations into the non-enzymatic origin of the citric acid cycle. J. Tennessee Acad. Sci. **57**, 48–50.
16. Schafer, T., Schonheit, P. (1991). Pyruvate metabolism of the hypothermophilic archaebacterium *Pyrococcus furiosus*. Arch. Microbiol. **155**, 366–377.
17. Shimoyama, A., Shigematsu, R. (1994). Dicarboxylic acids in the Murchison and Yamato-791198 carbonaceous chondrites. Chem. Letters **7**, 523–526.
18. Waddell, T. G., Henderson, B. S., Morris, R. T., Lewis, C. M., Zimmerman, A. G. (1987). Chemical evolution of the citric acid cycle: sunlight photolysis of 2-ketoglutaric acid. Origin of Life **17**, 149–153.
19. Waddell, T. G., Geevarghese, S. K., Henderson, B. S., Pagni, R. M., Newton, J. S. (1989). Chemical evolution of the citric acid cycle: sunlight and UV photolysis of cycle intermediates. Orig. Life Evol. Bios. **19**, 603–607.
20. Waddell, T. G. (1990). Addendum. Orig. Life Evol. Bios. **20**, 457.
21. Waddell, T. G., Miller, T. J. (1992). Chemical evolution of the citric acid cycle: sunlight photolysis of the amino acids glutamate and aspartate. Orig. Life Evol. Bios. **21**, 219–223.
22. Walton, R., Fraser-Reid, B. (1991). Studies on the intramolecular competitive addition of carbon radicals to aldehydes and alkenyl groups. J. Am. Chem. Soc. **113**, 5791–5799.
23. Weitzman, P. D. J. (1985). Evolution in the citric acid cycle. *In* Schleifer, K. H., Stackebrandt, E. (ed.), Evolution of Prokaryotes, FEMS Symp. nº 29, pp. 253–275. Academic Press, London, United Kigdom.
24. Willeford, K. O., Gibbs, M. (1989). Localization of the enzymes involved in the photoevolution of hydrogen from acetate in *Chlamydomonas*. Plant Physiol. **90**, 788–791.

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The use of functional inhibitors in the study of ribosomal evolution

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Summary

Forty different protein synthesis inhibitors with diverse kingdom and functional specificities have been used to measure the functional characteristics of different archaeal, bacterial and eukaryotic translational systems. This sensitivities data bank has been used to analyze functional correlations among the different ribosomal systems studied. The phenetic clusters obtained using functional analysis resemble the phylogenetic clusters generated by 16S/18S rRNA sequence comparison. These results strongly suggest that comparative functional analysis of an appropriate evolutionary clock, such as the ribosome, is of intrinsic phylogenetic value. Moreover, we postulate that the intersection between genotypic and functional phenotypic information, which we refer to as the functiotype, can facilitate the study of the evolution of the protein biosynthesis machinery.

Key words: protein synthesis, antibiotics, 16S/18S rRNA, phylogeny, functional evolution

Resumen

Se han utilizado cuarenta inhibidores de la síntesis proteica para determinar las características funcionales de sistemas de traducción arqueanos, bacterianos y eucariotas. La información generada se ha utilizado para analizar las correlaciones funcionales entre los sistemas ribosómicos estudiados. Las agrupaciones genéticas obtenidas se asemejan a las agrupaciones filogenéticas generadas por la

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comparación de las secuencias 16S/18S rRNA. Los resultados sugieren que el análisis funcional comparativo de un reloj evolutivo, como es el ribosoma, posee un valor filogenético intrínseco. Además, postulamos que la intersección entre la información genotípica y fenotípica funcional (el funcio-tipo) puede facilitar el estudio de la evolución de la maquinaria biosintética protéica.

Introduction

The introduction of new technologies such as gene cloning and fast sequencing of nucleic acids has resulted in important advances in the studies of evolution. Although the idea of using genotypic information stored in the primary sequence of macromolecules was developed by Zuckerkandl and Pauling forty years ago (25), the first attempts were done comparing the amino acidic sequences of singular proteins like cytochromes (9). The development of molecular evolution as a field had to wait for the establishment of reliable methodologies which allowed comparative gene sequencing of adequate evolutionary clocks.

The pioneering work of Carl Woese and coworkers using oligonucleotide catalogues of rRNAs led to the discovery of archaea (formerly named archaeobacteria) and provided the tools for the establishment of phylogeny based on the comparison of rRNA (22). These comparisons are based on the assumption that semantophoretic molecules are good evolutionary clocks because they are universal, their evolutionary rates are slow and the information they contain can be easily obtained (20). The phylogenetic value of this technique, however, is based on the assumption that changes in the primary sequence(s) are the result of the accumulation of mutations at a constant rate throughout evolution which is inconsistent with current knowledge of evolutionary biology.

Phylogenetic analysis using rRNA sequence comparison have also been used to establish the secondary structure of different rRNAs (23), to identify parts of the sequence which are universally conserved or characteristic of a group of organisms (denominated signatures, with high taxonomic value) or hypervariable. The hypervariable regions of the rRNA sequences are generally associated with promoters of secondary structure (double helixes), for which the only requirement is structural stability entailing complementary mutations for the maintenance of base pairing.

In this context, it is important to emphasize that while universal conservation of rRNA sequences has been associated with structures committed to function (12), the results of several point directed mutagenesis experiments remain ambiguous. Although the introduction of specific mutations into universally conserved rRNA sequences are in general lethal, complementary in vitro reconstitution experiments allowed the retention of functional ribosomal particles (8). This is an indication that assembly is a very critical step in the conformation of functional ribosomal particles. In order to facilitate this assembly some extremely conserved motifs are required to guide the complex intermolecular interactions. Point mutations in these guiding motifs do not allow assembly to proceed under physiological conditions. Nonphysiological in vitro reconstitution procedures, however, can promote the formation of fully active ribosomal particles, demonstrating that the mutation does not affect the functional part of the structure but only its assembly. Although limited information is available to prove this assumption, it is a very reasonable explanation for these rather controversial results.

Genotype versus phenotype

There are several algorithms which allow the establishment of phylogenetic relationships using rRNA sequences (10). Although it is undeniable that this type of analysis is very important in contemporary studies of evolution, there are several problems that have to be considered in the interpretation of the results. Some of them are related to the variable rates of evolution of different rRNAs, the variable rates of evolution inside an rRNA molecule, and the existence of genes with different sequences in the same microorganism (chimeric organisms). Some criticisms come from the more classical evolutionary schools such as palaeontology, and are related to the lack of appropriate dating of molecular trees. As mentioned, one possible way to overcome this problem is to assume a constant rate of evolution for the molecule, which is difficult to defend given the importance of horizontal gene transfer phenomena in evolution. The dispute between the two evolutionary schools, the molecular and the palaeontological, has been resolved by each school ignoring the other. The molecular evolutionists insist that phenotypic comparative studies are misleading in evolutionary studies because many phenotypic properties are the result of complex interactions between different gene products, which in most cases do not represent the genotypic information responsible for their expression.

It is obvious that the reduction of a coordinated expression of different genes to a plus or minus in a matrix of comparative data is an oversimplification of possible use for taxonomical purposes but clearly deficient in evolutionary information. Fig. 1 tries to depict this situation: a given phenotypic property is the representation of a phenotypic space resulting from the coordinated expression of different genes scattered, in most cases, in different operons along the chromosome(s). Most phenotypic properties are void of phylogenetic value not because of the properties themselves but as a result of our inability to decompose these end results into their different components. Obviously, the comparison of the genetic information responsible for their expression is of phylogenetic value. In addition, some of the genes could be under environmental regulation resulting in a change in the phenotype (different phenotypic space) without any modification of the genotype (Fig. 1B). While the regulation of gene expression by environmental conditions is clearly a very important factor, it is still not known how it affects the rate of evolution by selection processes.

In this communication we are presenting a new approach to the study of the evolution of protein synthesis machinery, in which functional space rather than sequence space is analyzed using specific functional inhibitors (2, 4, 6). The functional "footprinting" of ribosomes using antibiotics is a very specific part of the phenotype suitable for phylogenetic analysis because it corresponds to basic universal functional structures which are indispensable and unaffected by growth conditions. We propose for this singular part of the phenotype the name of functiotype (Fig. 1C). Of course the translation apparatus is not the only component of the functiotype, other basic cellular functions like transcription, replication, energy yielding processes, etc. could also have similar evolutionary implications. The advantage of protein synthesis over other systems is the outstanding body of structural, functional and genotypic information available resulting from its cellular abundance. Particularly useful is the wealth of inhibitors capable of interfering with protein biosynthesis, which facilitates the dissection of this rather complex cellular function (19).

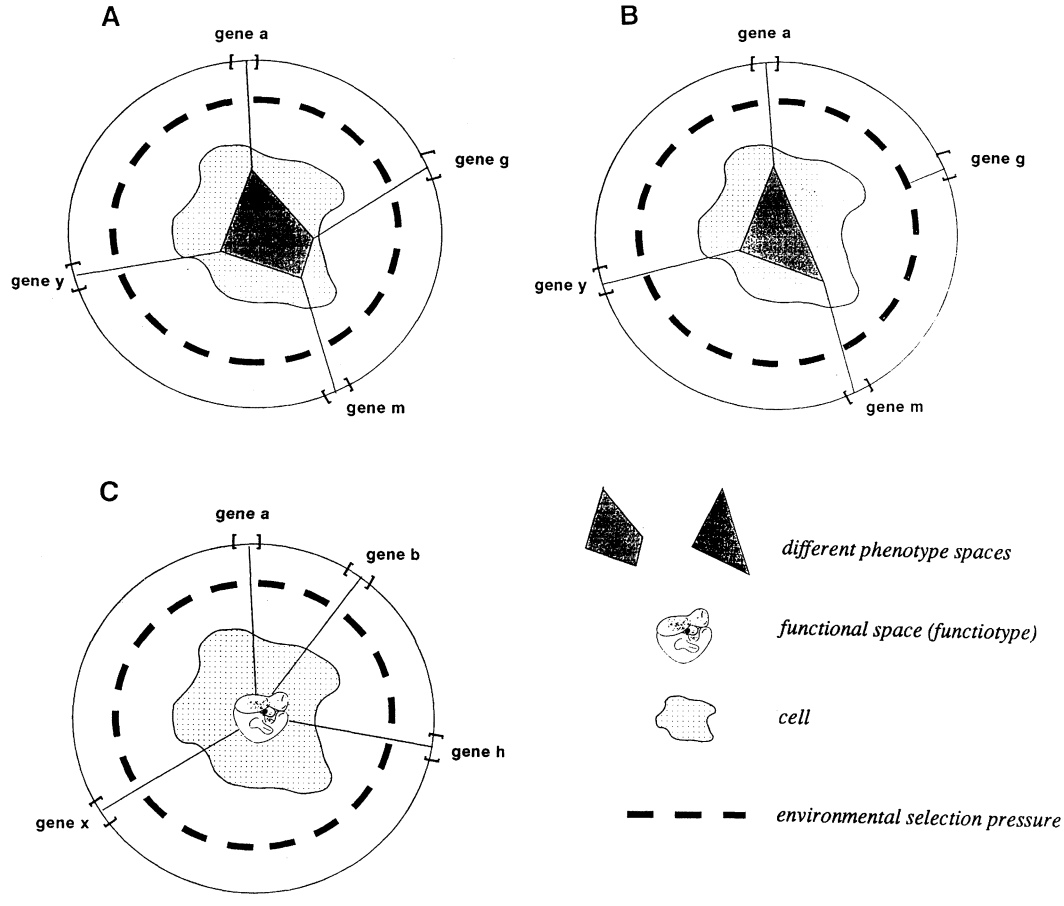

FIG. 1. Complexity of the analysis of the phenotypic space and model representation of the functional space. (A) The phenotypic space is represented as the area of the cellular space defined by the coordinated expression of the genes involved. The genotype has been ideally represented as an extracellular circle on which the genes are aligned. The control of environmental factors on gene expression has been represented as a selective screen. Depending on their position some of the genes are not expressed and the corresponding phenotypic space is therefore altered (B). (C) The functional space (functiotype) is represented using the same diagram. In the present work the functional space corresponds to the translational apparatus. The phylogenetic analysis of this functional space is based on the sequence comparison of only one of the genes responsible for its structure, the 16S/18S rRNA (gene b).

Inhibitors of protein synthesis as functional markers

During the last ten years our group has been collecting data on in vitro protein synthesis sensitivity to antibiotics specific to different structures, functions and domains. The sensitivity databank contains

the inhibitory effects of forty antibiotics on more than thirty ribosomal systems belonging to the three domains (kingdoms), bacteria, archaea and eukaryotes (4, 6). The inhibitors have been selected to represent the most important structural groups, the different functional specificities involved in the elongation step of protein synthesis (interaction of the ternary complex, peptidyltransferase and translocation) and the specificity exhibited towards different domains, i.e. specific inhibitors for bacterial ribosomes (type I), specific inhibitors for eukaryotic ribosomes (type II) and universal inhibitors (type III) (reviewed in 19). Specific inhibitors for archaea can not be considered since they have not yet been characterized.

Protein synthesis inhibition was tested in call-free systems to avoid interferences produced by transport and inactivation. Poly(U) mRNA was used to facilitate the comparison of sensitivities of

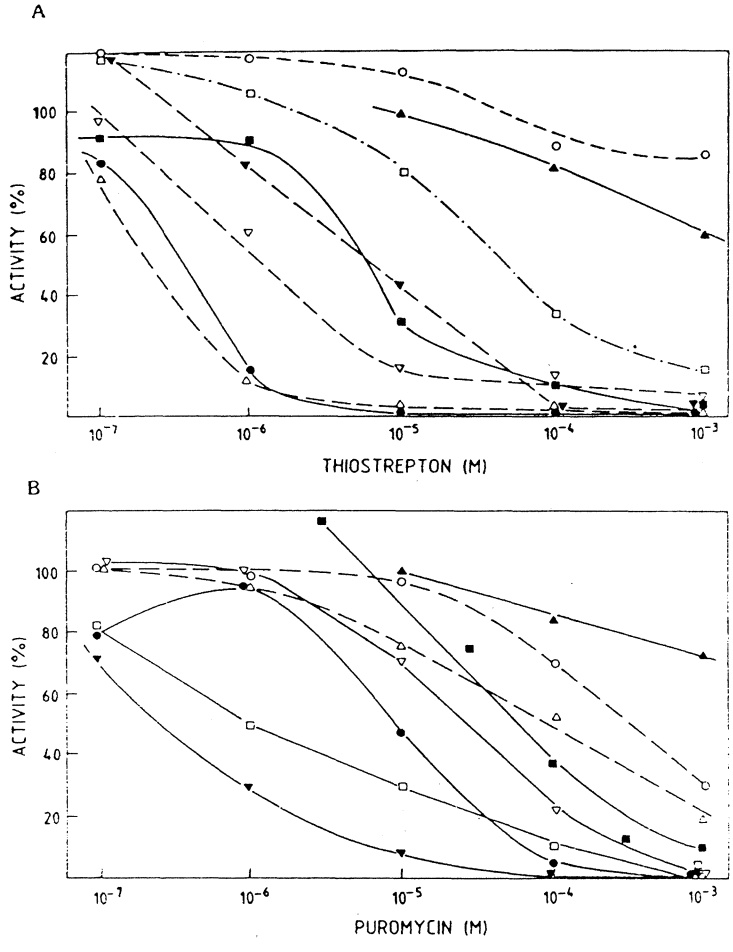

FIG. 2. Protein synthesis inhibition produced by thiostrepton and puromycin on different cell-free systems. (A) Thiostrepton: *S. cerevisiae; E. coli; M. formicicum; M. vanniellii; S. solfataricus; D. mobilis; H. salinarium; H. gibonsii.* (B) Puromycin: *S. cerevisiae; E. coli; H. halobium; H. marimortui; H. californiae; S. solfataricus; H. salinarium; M. thermoautotrophicum.*

ribosomal systems operating at very different conditions. The first requirement for this type of analysis is to obtain efficient in vitro protein synthesis systems. Once the conditions for protein synthesis for a specific system have been optimized, the inhibitory effect produced by different concentrations of antibiotics is measured. Fig. 2 shows the inhibitory curves of two antibiotics for different ribosomal systems, thiostrepton (specific inhibitor for bacterial ribosomes) and puromycin (universal inhibitor). As expected thiostrepton, a specific inhibitor of translocation, shows highly efficient inhibition of all the bacterial systems assayed but does not interfere with eukaryotic systems. In the case of puromycin, all the ribosomal systems studied are inhibited. Although this structural analog of aa-tRNA, is a universal inhibitor, the variations in its efficiency (Table 1), reflect the structural changes acquired by the peptidyltransferase centers as the result of their divergent evolution.

We have concentrated our analysis on archaeal systems in order to clarify their functional relatedness with the classical bacterial/eukaryotic systems. The conclusion, after analyzing more than twenty archaeal ribosomal systems belonging to the main groups; halophiles, methanogens, and sulphur-metabolizing archaea, was that archaeal ribosomes do not exhibit a bacterial or eukaryotic sensitivity pattern, but a mosaic of sensitivities probably related to their phylogenetic position. Some archaeal ribosomes, like those from the sulphur-metabolizing archaea, show extreme insensitivity to all known protein synthesis inhibitors (7, 17). This insensitivity is not due to the high temperatures required for their protein synthesis, since external controls using extreme thermophilic bacteria of the genus *Thermus* showed a pattern of sensitivity similar to that of the mesophilic reference bacteria *Escherichia coli* (7). This control strongly suggests that domain specificity (evolution) rather than ecological constrains (temperature) is responsible for the inhibitory patterns observed for sulphur metabolizing archaea (17). Methanogens exhibited the most variable range of sensitivities, probably a reflection of their broad phylogenetic diversity (3). The sensitivities measured for the halophilic archaea exhibited a similar mosaic pattern, although the near-saturation salt concentrations in which these systems operate raises doubts about the negative inhibitory values observed for cationic aminoglycoside antibiotics (18). Elaborate controls performed with the halotolerant bacteria *Vibrio costicola*, with ribosomes capable of performing protein synthesis at low and high ionic strength, suggest that competition rather than the absence of binding sites is responsible for the lack of inhibition of halophilic ribosomes by aminoglycosidic antibiotics (Marín and Amils, personal communication).

We used cluster analysis and reduced the sensitivity databank to a matrix in which similar sensitivities to reference systems, *E. coli* for bacteria and *S. cerevisiae* for eukaryotes, were scored as 1, while less sensitivity than the reference systems was scored as 0. The analysis of the matrix using conventional clustering programs showed strong functional correlations between systems related phylogenetically (13). Furthermore, the wealth of information stored in each inhibitory curve was preserved by means of a formula that transformed the sensitivity of each microorganism to different antibiotics into a dimensionless quantitative value (5). Table 1 gives some of the quantitative inhibitions calculated from inhibition curves for different antibiotics and cell-free systems. The phenetic clusters generated (Fig. 3) are very similar to the phylogenetic trees obtained using total sequence comparison of 16S/18S rRNAs (21, 24).

TABLE 1. Quantitative matrix data of the sensitivities of archeal, bacterial and eukaryotic cell-free systems to different protein synthesis inhibitors

	Methanobacterium formicicum	*Methanococcus vanniellii*	*Sulfolobus solfataricus*	*Desulfurococcus mobilis*	*Thermococcus celer*	*Thermoplasma acidophilum*	*Halobacterium salinarium*	*Halococcus morrhuae*	*Natronococcus occultus*	*Escherichia coli*	*Bacillus stearothermophilus*	*Vibrio costicola*	*Saccharomyces cerevisiae*
Althiomycin	278	711	0	0	0	30	466	112	296	1142	1342	1147	74
Carbomycin	616	691	0	0	0	42	624	293	580	1280	1505	1312	72
Griseoviridin	209	250	0	112	0	0	649	112	0	1938	1020	1872	248
Thiostrepton	1098	1829	192	616	1780	486	1316	892	1758	1834	1644	1158	64
Tylosin	513	372	0	0	96	169	114	85	174	1603	1922	2048	6
Viomycin	1422	169	0	48	182	268	248	0	113	2002	1757	1358	105
Virginiamycin M	281	362	244	436	284	255	83	27	0	1668	1738	2370	206
Anisomycin	485	0	0	0	0	0	776	367	544	0	0	228	1025
Bruceantin	694	345	0	0	90	0	74	–	–	7	0	0	2025
Cycloheximide	0	0	0	0	0	0	0	0	0	0	327	0	1391
Streptimidone	0	30	0	0	0	84	81	0	0	48	218	96	766
Streptovitacin A	0	30	0	0	0	0	105	0	0	45	0	60	824
Haemanthamine	0	0	0	0	0	0	31	0	0	306	0	0	888
Harringtonine	0	0	0	0	0	0	130	0	0	188	115	150	762
Mitogillin	120	125	211	544	225	614	100	0	0	584	296	168	1428
Narciclasine	1496	45	0	240	60	129	186	0	0	0	70	0	1691
Pretazetine	60	0	0	0	0	0	0	0	0	170	390	25	812
Restrictocin	300	340	158	533	200	702	150	0	0	570	184	512	1394
Alpha-Sarcin	736	723	409	1066	195	548	90	0	0	579	136	190	1871
Toxin T2	130	125	0	0	0	0	21	0	0	0	475	55	1590
Tylophorine	130	0	0	0	0	70	0	0	0	138	350	100	1142
Tubulosine	66	0	0	0	0	15	0	0	0	72	434	0	1091
Amicetin	0	41	0	0	0	69	537	126	105	1078	1078	968	156
Anthelmycin	1138	181	228	0	92	230	900	276	217	968	1035	1000	1078
Blasticidin S	2238	414	0	66	0	0	578	724	676	1608	467	1954	1992
Edein	645	315	755	90	1229	686	98	112	110	1292	892	2172	1668
Sparsomycin	1167	732	547	30	0	348	1502	1068	1454	998	1378	1394	1026
Higromycin B	1438	196	57	0	0	216	36	54	233	1545	474	1458	1354
Puromycin	448	920	105	298	456	187	1792	493	1322	589	546	808	350
Tetracycline	507	183	114	79	224	213	206	202	303	926	1115	1139	782
Fusidic acid	878	989	0	0	30	60	189	119	24	557	1056	908	1055

The sentivities for the different ribosomal systems have been obtained from references 1, 7, 16, 18 and Amils and cols. (unpublished work), and transformed into quantitative data using the algorithm described in ref. 5.

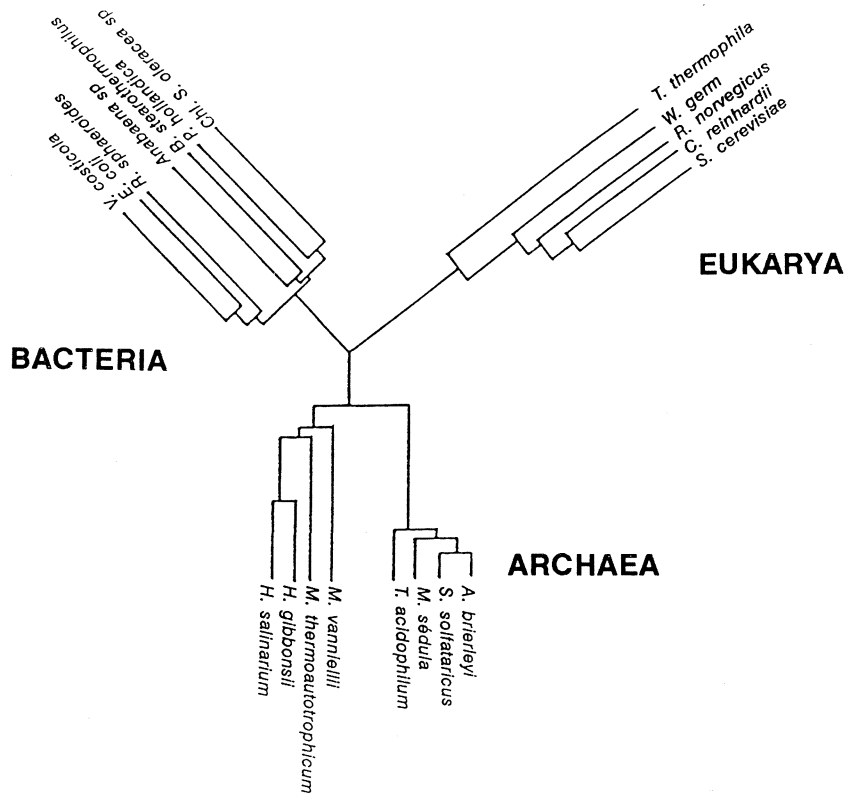

FIG. 3. Phenetic tree obtained by using quantitative antibiotic sensitivity data.

Functional inhibitors as phylogenetic markers

The finding that different ribosomal systems, with adequate evolutionary distance, exhibited a gradual change in sensitivities to different antibiotics gives rise to the idea that our databank was sensing the functional evolution of ribosomes. Fig. 4 shows a model representation of the type of analysis that we are performing using protein synthesis inhibitors. The functional ribosomal space is a multiple gene product combining rRNA and rproteins genes, in which different nucleotides from different genes cooperate in the structural requirements for the different ribosomal functions. A specific inhibitor for a ribosomal function corresponds to a molecule which can interfere with the functional space by means of a direct interaction. When a point mutation in one of the genes alters part of the functional structure without interfering with the ribosomal function, it can affect the affinity for the antibiotic, thus producing a less sensitive, an insensitive, or an even more sensitive ribosome. In the model presented in Fig. 4 a point mutation in nucleotide E of gene h affects the interaction of the functional probe (antibiotic), thus producing an insensitive ribosome without affecting the ribosomal function. Mutations affecting ribosomal function will not be selected for. The collection of differential sensitivities exhibited by

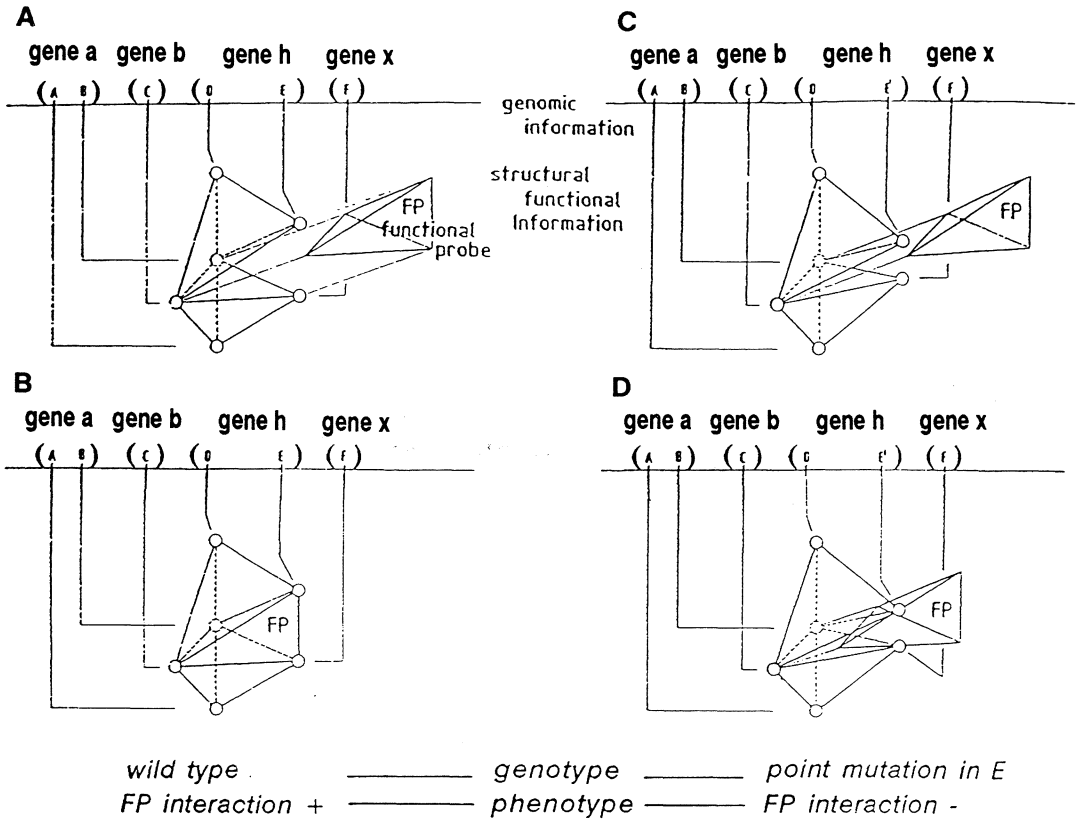

FIG. 4. Model representation of the correlation between ribosomal genotypic information and functional phenotypic information detected by the functional probes (antibiotics) used in this work. The genomic information represents those nucleotides in the genes which are responsible for the structural functional information (functional space) detected by a functional probe (antibiotic) (A and B). A point mutation in nucleotide E of *gene h* produces a structural change in the functional space which as a result is unable to interact with the functional probe (C and D).

ribosomes belonging to different domains (kingdoms) are the result of the natural selection of different mutations which drive the evolution of the ribosomal particles. Obviously there are limitations to this type of analysis, the most important being the lack of knowledge regarding the amount of functional space footprinted by the selected antibiotics, but they are of the same magnitude as those imposed by the comparative sequence analysis of only one ribosomal component, for instance 16S/18S rRNA, which is a disrupted representation of the ribosomal functional space.

The existence of antibiotic binding sites maintained in all systems strongly supports the idea that the basic components of the translational machinery have been preserved throughout evolution. In principle, phylogenetically shared sensitivities should either antedate the radiation of the three lineages of descent or have come into existence by convergent evolution. At present, it seems more reasonable

to assume that phylogenetically shared sensitivities represent primeval features of a common ancestral progenote that were retained by any two or all three lines of descent. In this context, we can argue that antibiotic sensitivity differences displayed by archaea, in contrast to the uniform pattern exhibited by bacteria and eukaryotes, indicate that archaea lost homology with one another by virtue of the differential acquisition or loss of their sensitivities, whereas bacteria and eukaryotes did not.

This conclusion implies that the early evolution of archaea must have been fundamentally different from that of the other two lines of descent. One possibility is that the common archaeal ancestor split into different sublines very early, before stringent genetic integration imposed severe restrictions on the possibility of changes. Others are that archaeobacterial ribosomes represent either alternate solutions for the same function or diverse states of evolution of ribosomal structures not yet optimized due to the lack of competition in the ecologically extreme habitats in which they developed. Of course, we can not ignore the fact that the current lack of specific inhibitors for archaea could be responsible for this pattern.

Once the analysis of the functional data bank is refined by preserving the information provided by the inhibition curves, the homogeneous patterns of sensitivities described for the bacterial and eukaryotic ribosomes disappear. The full spectrum of bacterial and eukaryotic diversity has not yet been explored. In addition, the possibility of finding bacterial and eukaryotic organisms with heterogeneous patterns of sensitivity is illustrated by the recently described extremely thermophilic, anaerobic bacteria, *Thermotoga maritima*, which on the basis of 16S/18S rRNA sequence homology is the bacterium closest to the diversification point of the phylogenetic tree (24) and which correspondingly exhibits an abnormal pattern of sensitivity to aminoglycoside antibiotics (11).

We still do not have a clear picture of the ribosomal prototype that gave rise to the existing ribosomal systems, although if we compare the current situation with the ideas discussed a few years ago (6), we see significant progress. Once the phylogenetic consistency of the functional inhibitory data bank is established, it can be used to generate and discard functional models. It would be interesting to intersect the ribosomal sequence space (genotype) with the correspondent functional space (functiotype) in order to increase the amount of information on the molecular bases of ribosomal function and its evolution. As a first step and in collaboration with other groups we are using comparative rRNA footprinting studies to generate information on specific nucleotides involved in antibiotic binding with the correspondent specific function inhibited (14). However, several examples of differences in antibiotic sensitivity in ribosomes with no apparent differences in the rRNA sequence suggest that functional sites depend on cooperative interactions between rRNAs and rproteins (15). This fact complicates the analysis since to date there are no appropriate techniques with which to analyze ribosomal proteins at the same level of resolution as rRNA. There is no doubt that we need to face this problem if we want to understand the evolution of the translational apparatus at the functional level.

At any rate, the current state of the art indicates that relevant information on the functional structures of the protoribosome can be obtained from the antibiotic sensitivity spectra of contemporary ribosomes. This may allow some of the fundamental questions about the evolution of the translational apparatus to be addressed.

References

1. Altamura, S., Sanz, J. L., Amils, R., Cammarano, P. (1988). The antibiotic sensitivity spectra of ribosomes from the Thermoproteales. Phylogenetic depth and distribution of antibiotic binding sites. Syst. Appl. Microbiol. **10**, 218–225.
2. Amils, R. (1993). Functional phylogeny. *In* Guerrero, R., Pedrós-Alió, C. (ed.), Trends in Microbial Ecology, pp. 563–566. SEM, Barcelona, Spain.
3. Amils, R., Londei, P., Cammarano, P. (1993). Translation in Archaea. *In* Matheson, A. T., Kates, M., Kushner, D. J. (ed.), The Biochemistry of Archaea (Archaebacteria), pp. 393–438. Elsevier, Amsterdam, Netherlands.
4. Amils, R., Ramirez, L, Sanz, J. L., Marín, I., Pisabarro, A. G., Sánchez, E., Ureña, D. (1990). Phylogeny of antibiotic action. *In* Hill, W., Dahlberg, A., Garret, R. A., Moore, P. B., Schlessinger, D., Warner, J. R. (ed.),The Ribosome: Structure, Function and Evolution, pp. 645–654. ASM, Washington, DC.
5. Amils, R., Ramirez, L., Sanz, J. L., Marin, I., Pisabarro, G., Ureña, D. (1989). The use of functional analysis of the ribosome as a tool to determine archaebacterial phylogeny. Can. J. Microbiol. **35**, 141–147.
6. Amils, R., Sanz, J. L. (1986). Inhibitors of protein synthesis as phylogenetic markers. *In* Hardesty, B., Kramer, G. (ed.), Structure, Function and Genetics of Ribosomes, pp. 605–620. Springer Verlag, New York, NY.
7 Cammarano, P., Teichner, A., Londei, P., Acca, M., Nicolau, B., Sanz, J. L., Amils, R. (1985). Insensitivity of archaebacterial ribosomes to protein synthesis inhibitors. Evolutionary implications. EMBO J. **4**, 811–816.
8. Cunningham, P. R., Weitzmann, C. J., Negre, D., Sinning, J., Frick, V., Nurse, K., Ofengand, J. (1990). In vitro analysis of the role of rRNA in protein synthesis: site-specific mutation and methylation. *In* Hill, W., Dahlberg, A., Garret, R. A., Moore, P. B., Schlessinger, D., Warner, J. R. (ed.), The Ribosome: Structure, Function and Evolution, pp. 243–252. ASM, Washington, DC.
9. Fitch, W. M., Langley, C. H. (1976). Protein evolution and the molecular clock. Fed. Proc. **35**, 2092–2097.
10. Gouy, M., Li, W. (1990). Evolutionary relationships among primary lineages of life inferred from rRNA sequences. *In* Hill, W., Dahlberg, A., Garret, R. A., Moore, P. B., Schlessinger, D., Warner, J. R. (ed.), The Ribosome: Structure, Function and Evolution, pp. 573–578. ASM, Washington, DC.
11. Londei, P., Altamura, S., Huber, R., Stetter, K. O., Cammarano, P. (1988). Ribosomes of the extreme thermophilic eubacterium *Thermotoga maritima* are uniquely insensitive to the miscoding inducing action of aminoglycoside antibiotics. J. Bacteriol. **170**, 4353–4360.
12. Noller, H. F. (1984). Structure of ribosomal RNA. Annu. Rev. Biochem. **53**, 119–162.
13. Oliver, J. L., Sanz, J. L., Amils, R., Marín, A. (1987). Inferring the phylogeny of archaebacteria: the use of ribosomal sensitivity to protein synthesis inhibitors. J. Mol. Evol. **24**, 281–288.
14. Rodriguez-Fonseca, C., Amils, R., Garret, R. (1995). Fine structure of the peptidyl transferase centre on 23 S-like rRNAs deduced from chemical probing of antibiotic-ribosome complexes. J. Mol. Biol. **247**, 224–235.
15. Sánchez, E., Teixidó, J., Guerrero, R., Amils, R. (1994). Hypersensitivity of *Rhodobacter sphaeoides* ribosomes to protein synthesis inhibitors. Structural and functional implications. Can. J. Microbiol. **40**, 699–704.
16. Sanz, J. L., Altamura, S., Mazziotti, I., Amils, R., Cammarano, P. (1987). Unique antibiotic sensitivity of an "in vitro" polypeptide synthesis system from the archaebacterium *Thermoplasma acidophilum*. Phylogenetic implications. Mol. Gen. Genet. **207**, 385–394.
17. Sanz, J. L., Huber, G., Huber, H., Amils, R. (1994). Using protein synthesis inhibitors to establish the phylogenetic relationships of the Sulfolobales order. J. Mol. Evol. **39**, 528–532.
18. Sanz, J. L., Marín, I., Ureña, D., Amils, R. (1993). Functional analysis of seven ribosomal systems from extremely halophilic archaea. Can. J. Microbiol **39**, 311–317.

19. Vázquez, D. (1979). Inhibitors of protein biosynthesis. *In* Kleinzeller, A., Springer, G. F., Wittmann, H. G. (ed). Springer-Verlag, Berlin, Germany.
20. Woese, C. R. (1982). Archaebacterial and cellular origins: an overview. Zbl. Bakt. Hyg. I. Abt. Orig. **C3**, 1–17.
21. Woese, C. R. (1987). Bacterial evolution. Microbiol. Rev. **51**, 221–271.
22. Woese, C. R., Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci. USA **74**, 5088–5090.
23. Woese, C. R., Gutell, R., Gupta, R., Noller, H. F. (1983). Detailed analysis of the higher order structure of 16S-like ribosomal ribonucleid acids. Microbiol. Rev. **47**, 621–669.
24. Woese, C. R., Kandler, O., Wheelis, M. L. (1990).Towards a natural systems of organisms: proposal for the domains Archaea, Bacteria and Eucarya. Proc. Natl. Acad. Sci. USA **87**, 4576–4579.
25. Zuckerkandl, E., Pauling, L. (1965). Molecules as documents of evolutionary history. J. Theor. Biol. **8**, 357–366.

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The unresolved enigma of the origin of life: at the centennial of the birth of Aleksandr I. Oparin

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Summary

Nowadays we live the age of microorganisms, without which life on Earth would not exist. Some essential processes are carried out by one only group of microbes, and only a small fraction of extant microbes have been studied. Well-established microbial communities might have existed already 3500 million years ago, whereas humans are new comers to the biosphere. We consider ourselves to be the center of a planet that started without us, and that probably would go on without us. The fundamental problem when we try to explain the origin of life is the discussion of conditions and processes very distant in time, which happened in environments unknown to us. In 1924, Oparin published a book which focused the study of the origin of life in a materialistic perspective. The idea of the single act of spontaneous generation was thus replaced by a step-wise process of chemical evolution, with different of growing complexity. The best homage to Oparin is to continue the discussion of his ideas adding new doubts and questions.

Key words: origin of life, Oparin, chemical evolution, origin of cell, earliest microorganisms

Resumen

Nos encontramos en plena era de los microorganismos, sin cuya existencia la vida en la Tierra no sería posible. Hay procesos esenciales desarrollados exclusivamente por un tipo de microbios y, de todos

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los actualmente existentes, sólo se ha estudiado una pequeña fracción. Algunas poblaciones microbianas tienen 3500 millones de años, mientras que los humanos puede decirse que acaban de arribar a la historia de la vida. Nos consideramos el centro de un planeta que empezó sin nosotros y que sin nosotros podría continuar. El problema fundamental cuando se trata de explicar el origen de la vida es la referencia a procesos lejanos en el tiempo que tuvieron lugar en un medio que nos es desconocido. En 1924, Oparin publicó un libro que se acercaba al origen de la vida desde una perspectiva materialista. La idea de un solo acto de generación espontánea fue reemplazada por un proceso de evolución química, con diferentes niveles crecientes de complejidad. El mejor homenaje que se puede hacer a Oparin es proseguir la discusión de sus ideas añadiendo nuevas dudas y misterios.

The age of microorganisms

Many people tend to believe that the Earth was designed to satisfy the needs of the human species, which would have been the culmination of a progressive evolution directed to achieve goals of excellence, such as intellectual abilities. People think also that we live the age of technology, in which technoscientific civilization can dominate everything. A wider vision, which had taken into account the whole richness of fauna, either known or inferred, would make us believe that we live the age of arthropods or even the age of insects, regardless of the acceptance or not of the Darwinian paradigm of human insignificance, and even if we were conscious that we are just another species inhabiting the Earth.

In fact, insects are the organisms among the animals with the widest diversity of species, ecosystems, and survival and behavior strategies. However, we could still expand our perspective and consider the whole biosphere, the flux of chemical compounds and energy at a planetary scale. Then we would reach the conclusion that we actually live the age of microorganisms, and that, without them, terrestrial life, as we know it, would not exist (24). Some essential processes, such as the incorporation of atmospheric nitrogen to the inventory of biogenic elements, can be carried out only by a given group of microorganisms.

The biochemical virtuosism of bacteria is amazing, and we have studied only a very small fraction of extant microbial species. Moreover, palaeontological studies confirm that diversity is very old. We know that the Earth is about 4550 million years old and well-established microbial communities might have existed 3500 million years ago, probably not very different from modern microbial mats (39). In any case, using arguments taken from chemistry and bacterial genetics, it seems reasonable to suppose that the transit from organic matter to the first microbial communities took place in a short period of time, let us say some 10 million years (23). Besides, we must accept the fact that the genus "Homo" emerged as a different branch from the rest of primates just 2 million years ago. That is to say, we have appeared on the Earth at the recent moment in the history of life. Yet, we believe that we are the central and principal part of a planet that, as a matter of fact, was made without us and that probably will go on without us.

Processes and principles of the origin of life

In sharp contrast to phenomena related to physics or chemistry, in biology things are the way they are for historical reasons. A deep comprehension of biologic processes will not be achieved without a previous knowledge of their origin and development. This is a viewpoint that finds its roots in the philosophy of Heraclitus of Ephesus, and on which authors with such different ideas as Theodosius Dobzhansky, Ernest Mayr and Aleksandr I. Oparin have insisted. The acceptance of this principle among biologists is not general. However, from the seminal contribution of Darwin, who showed that evolution was a real fact, and that causal explanations of the phenomenon—natural selection—could be given, all biological sciences have been more and more interested in evolutionary studies.

The basic problem when trying to explain the origin of life is the reference to processes so distant in time, which took place in environments unknown to us. Moreover, we have witnessed one only kind of life—the terrestrial one—of which we have just a rudimentary knowledge. We will focus on an irreproducible, complex historical fact: the formation of early microbial communities, which evolved from more elementary cells, which in turn had originated from the simplest cellular blueprints, in terms of biochemical functions. We biologists can easily deal with the matter of cellular evolution, for all of us are aware—and accept it—that we have come from a single cell. The insolvable riddle is the abyss still existing between the most sophisticate mixture of chemical compounds and the simplest cell that can be imagined. We will never know what really happened at the beginning (28, 37).

In his book on the origin of species, Darwin had resorted to a divine contribution to justify the emerging of the earliest forms of life (compare the last paragraph of «The Origin of Species», second edition—1860—and later editions, with the text in the first edition). However, he had privately confessed that it had been just a way to avoid a topic which he could not discuss due to the lack of data. In 1871, in a letter addressed to the botanist J. D. Hooker, Darwin suggested that life could have originated in a small temperate pond, abundant in chemical compounds that, exposed to different energy sources, such as light, heat and electricity, would have formed the first "proteic compounds". In any case, Darwin was far from basing his theory on speculative basis about unsupported and unknown reasons.

Nevertheless, scientists like Haeckel or Huxley had favoured the idea that the properties of life might have appeared as soon as the chemical complexity had allowed it, even without any cellular structure. This provoked temporary enthusiasm for spontaneous generation, which totally disappeared when, in the 1860's, Louis Pasteur showed that not only worms or rats, nor even microbes could emerge from inert substances or objects. The development of the cellular theory—particularly Virchow's postulate: "omnis cellula e cellula"—meant an end to the belief in a spontaneous origin of life. The cellular theory established that the properties of life came from a previous cellular organization.

Simple chemical activity—complex as it might be—could have never generated the complex structure of a cell—simple as it might be. Thus, 19th century science would never admit the possibility of life spontaneous generation under any natural condition. Evolutionists lacked a plausible explanation for the beginning of the evolutive process. To avoid the problem of how life originated on Earth, some resorted to spores or seed from unknown origin, which would have come from outer space, which is what the panspermia theory postulates. The physical distance of the phenomenon would have made no possible its scientific study. F. Engels, based in Haeckel's ideas, in his book «Dialectics of Nature»,

suggested a materialist basis for the riddle of the origin of life, which refused spontaneous generation, vitalism and, especially, a divine intervention in the evolutionary process.

Oparin: the prebiotic soup hypothesis

In 1924, the Russian biochemist Aleksandr I. Oparin published a short book which dealt with the problem of the origin of life in a materialist way. «The Origin of Life» (31). A more elaborated and extensive book was published in English in 1938—the first edition, in Russian, had been published in 1936. It was then that Western scientists had access to his points of view: the previous idea of a single act of spontaneous generation had been replaced by a process of chemical evolution, with different levels of growing complexity. Besides extending the rank of the evolutionary thinking, this idea gave renewed support to Darwinism. In fact, Oparin suggested that natural selection could have acted as a mechanism of optimization among pre-biotic structures.

In 1929 the British biologist J. B. S. Haldane suggested, independently from Oparin, that viral particles had originated from organic matter (10). According to the initial proposals of Oparin, primitive cells would have been the result of a crucial and fortuitous combination of different chemical compounds. The innovation that Oparin introduced in later contributions was to give up the idea of a sudden origin of life, that had taken place in one only step, from improbable, random chemical combinations (21). He suggested a very elaborated model without no clear boundary between life and non-life. Chemical evolution would gradually increase the level of organization to give a continuity between inert matter and the first living cells.

In 1977, John Farley (8) suggested that Oparin's vision might have changed under the influence of dialectical materialism—Engels' work had been published in 1925—and he pointed out that the success of Oparin's work provided a historical counterweight to the disaster produced by the anti-scientific madness promoted by T. D. Lysenko in the Soviet Union. Lysenko introduced Lamarckism—the theory that asserted that structural changes caused by environmental changes were transmitted to offspring—in the official Soviet philosophy of the thirties. Taking into account that the letter written by Darwin to Hooker was published in 1935, then historically there are at least four materialist explanations to the origin of life, which were formulated independently by Darwin, Oparin, Engels and Haldane. However it is to Oparin that we owe the richest, most fruitful vision of the origin of life, from a scientific and intellectual point of view.

A short explanation of the origin of life, according to Oparin, must begin with the consideration of the chemical composition of the early atmosphere. For many years, it was generally believed that those gases were the remains of the solar nebulae from which all planets originated. Most of them would be highly reactive, chemically reduced compounds—rich in electrons—such as methane, ammonia or hydrogen, and oxygen would be absent. The joint action of different energy sources—lightning, heat, radiation—would have subjected the atmospheric gases to chemical transformations that would have resulted in a group of organic compounds, which would progressively enrich the oceans. A primordial broth, or soup, would have originated, which would be the starting point for later complex organization processes, beginning with rudimentary polymers, followed by aggregates with the first attributes of

living matter and ending up with primordial cells—probionts—, which would be, however, very different from even the simplest bacterium found today (37).

The Miller-Urey experiment... and its sequels

Events that took place after Oparin's proposals showed effectively that prebiotic synthesis of organic compounds is a process which can be reproduced in the laboratory. Oparin ideas linked to Urey's hypothesis on the chemistry of the early atmosphere were the starting point for the design of the historical experiment performed by Stanley L. Miller in 1953 (26) when he published a seminal article in which he described how organic compounds could be formed abiotically in the laboratory. Miller, by then a young graduate student working with Harold Urey, designed a experiment which is still known as the Miller-Urey experiment. He added a mixture of hydrogen, ammonia and methane to carefully purified and sterilized water and subjected all this to electrical discharges for a week. He found as products what turned out to be a complex mixture of organic compounds, such as organic acids, alcohols, and amino acids. From the experimental confirmation of some of the steps of the process which Oparin had already postulated, his vision of the origin of life became an essential component of modern evolutionary thought.

Since the 1960's, geochemists have proposed a dilemma based on the Oparinian model, and not yet solved. Today explanations of the origin of our solar system suggest that the early atmospheres of inner planets—Venus, the Earth and Mars—formed by reduced gases, were replaced by atmospheres formed by volcanic emanations. So, the most abundant chemical compound would be carbon dioxide. Simulation experiments show that, in that case, prebiotic organic synthesis would be negligible. Some authors have searched for alternative sources of organic matter. In the early 1960's Joan Oró (35) postulated that the Universe should be abundant in organic matter. Radioastronomical explorations have confirmed this theory: the most extended chemistry all around the Universe is organic. However, in terms of organic matter the Earth is located in the poorest region of the Solar System (25), which indicates that something more than carbon compounds must have been required for the origin of life.

It seems that one of the essential prerequisites of life is water in the liquid state. The Earth, throughout most of its history, has maintained all the conditions that favor the abundance of water on its surface. Mars surface is full of channels, and the erosive action of water has been detected. Aquatic environments may have occurred on that planet for only a few hundreds millions of years. The red planet might hide, still untouched, the chemical, palaeontological footprints of what might have been incipient life, which very soon must have disappeared due to the climatic consequences of the rapid declining of Martian geological activity: the dramatic decrease of temperature leading to permanently frozen water (25).

Oró (34) was also the first to suggest the possibility of a link between substances from comets and compounds involved in the origin of life. There is a remarkable correlation between the amino acids obtained in Miller's experiment and the chemical composition of some meteorites. Frequent impacts on the Earth by comets and other itinerant bodies from the outer space which contain organic compounds might have been an excellent source of essential predecessors responsible for the beginning of the earliest biochemical reactions. C. Chyba and C. Sagan (4) have suggested that all these could be possible

although some of the impacts on the planet may have had deletereous effects. We are not referring to collisions with relatively small objects—such as the one that is supposed to have provoked the extinction of dinosaurs some 65 million years ago, which might have had a 10 km diameter—, but to collisions with asteroids with catastrophic consequences, such as increasing the oceans' temperature up to the water boiling point, evaporating the oceans water and annihilating the early ecosystems (40).

The search of places where the most reactive matter is abundant has directed our attention to several kinds of terrestrial ecosystems discovered in the late 1960's, such as submarine hot springs—or deep sea vents. After J. B. Corliss' discovery of the deep sea vents near the Galapagos Islands, others have been studied along the friction line of tectonic plates. These are, in terms of biology, exceptional places both in faunistic abundance—most of the animals found there were totally unknown—and in ecological relationships. Communities which live in these depths do not directly depend on light, for these are completely dark ecosystems. However they depend on the energy originated from inorganic chemical reactions. Some authors have seen, in these hot springs, a model of early environments, where the synthesis of the compounds and ingredients needed for the origin of life could have developed (13).

The first autoreplicative molecule

Present-day living matter is organized in a network of complex interactions between molecules which have information (nucleic acids) and molecules which execute this information (proteins). At present it would be impossible to separate one action from the other. Both molecules are so intimately integrated that their respective functions depend on the presence of each other. Nobody could imagine a functional protein without a genetic message which perpetuated it, just as it is difficult to conceive stable information, with hereditary transmission, without proteins intervening in the process. In the view of this fact, one can wonder what the organization of the early living matter was like. Or reformulating the classic enigma, which came first, the chicken or the egg?, nucleic acids or proteins? The latest advances in biochemistry let us venture provisional answers to this dilemma. In 1981 the capability of RNA to catalyze chemical reactions was observed. Discoveries by Thomas Cech and Sidney Altman opened a window to the early Earth. They allow us to imagine what the first molecule with autoreplicative capability might have been like: an RNA which must have contained the necessary information for self-replication. We talk now of a «RNA World» which is a biosphere of creatures with a biochemistry based on the capabilities of those polymers. The RNA was already considered as the first genetic material in the early hipotheses formulated by Carl R. Woese (43), Francis H. C. Crick (5), and Leslie E. Orgel (32), but after the discovery of ribozymes the idea was further developed by Gilbert (9) —who coined the term «RNA World»—, Bruce M. Alberts (1) and Antonio Lazcano (20).

There are several facts which support the idea of an early life based on RNA as a precursor of life based on DNA (14,37): (i) RNA plays a crucial role in present-day cells; DNA genomes replication starts with RNA primers, and mRNA, tRNA and rRNA are indispensable ingredients for the translation; protein synthesis can take place in the absence of DNA, but not in the absence of RNA. As a matter of fact, peptidyl transferase, the enzymatic activity responsible of the peptide bond formation, is most probably of ribonucleic nature (30). (ii) Many coenzymes such as coenzyme A, NAD and ATP, are

ribonucleotides or have derived from them. (iii) Synthesis of the amino acid histidine needs the participation of both ATP and a derivative of ribose. (iv) In present-day cells, the synthesis of deoxiribonucleotides—DNA precursors—takes place from ribonucleotides.

Problems, however, do not finish with this model. On the contrary they have just begun. RNA is an extremely complex molecule, with very small chances of having originated from spontaneous chemical processes in a prebiotic world. The nature of catalyzers and of different agents which must have favored the formation of the first polymers is a complete enigma for chemistry. Gerald Joyce and L. E. Orgel (16) have recently established the paradox by presenting the "RNA World" as "the dream of molecular biologists and the nightmare of prebiotic chemists". Currently there are different alternatives to imagine what the world previous to the formation of autoreplicative RNA would be like but not all of them are equally supported by experiments. They range from the model suggested by radicalist A. G. Cairns-Smith, who suggested the existence of mineral genes made of clay (3), to the kind of molecules that would look like nucleic acids but they were not—that is to say, eggs, but not hen's eggs (17,33). Nevertheless, some laboratories explore now the possibilities of RNA being a molecule able to evolve in test tubes. Jack Szostak has tried to obtain a catalytic RNA able to self-replicate (2,15). The strategy to do so consists of the establishment of chemical conditions which favor natural selection—at the molecular scale—of RNA molecules with well defined abilities and thus allowing the empirical exploration of the sequence space (7). From a biotechnological point of view, this approach will open the possibility to obtain macromolecules new properties with a strategy radically different to the traditional «rational design».

The transition from the «RNA World» to a «Protein-DNA World» would imply the invention of ribosome-based translation—with the establishment of a genetic code—and the substitution of DNA for RNA as the genetic material (22). The comparative biochemistry of the three major cell lineages — Bacteria, Eukarya, and Archaea (45)—shows that all those major transitions took place before the divergence from the last common ancestor.

The origin of cells

Many hypotheses on the origin of life overlook one fundamental aspect, i.e., that of the origin of compartments. In contrast to Haldane, Oparin pointed out the origin of cells as the crucial step in the emergence of life (21). Nowadays the knowledge derived from research on bioenergetics makes it clear that compartments surrounded by membranes are necessary for the transformation of energy. First, we must take into account a thermodinamic imperative, which is a prerequisite also in biological systems: without constant energy flux, it would not be possible to obtain—from unorganized matters—the overwhelming organization found even at the simplest stages of living matter. Besides, the chemiosmotic hypothesis formulated by Peter Mitchell in 1961 offers an explanation (27), a logical mechanism for what could be called "universal—and ancient—principles", from which all biological energetic machinery must have originated. There is a "chemiosmotic essence" of life: energy fluxes are inconceivable, from a biological point of view, in the absence of compartments (see ref. 11 for a thorough review). This is to say, there is life when there is a cell; cellular membranes are the boundaries to establish the difference between internal and external environment, which is essential to define living beings. Virchow's

mechanistic neovitalism, which considers cells to be the unity of life, is nowadays represented by the chemiosmotic corollary: cell is the simplest possible living being on this planet. The principle of continuity agrees with Oparin: in fact, the origin of cell must have been the crucial process. Unfortunately, we are completely ignorant of how this process took place. We have just a few models—and they are still poorly elaborated—for the study of both the origin of membranes and early cells (6,38).

Guy Ourisson has proposed the terpenoid theory for the origin of cells based in the observation that no terpenoid-free cellular membrane is known (36). Terpenoids of one type or another—polyprenic lipids in Archaea, hopanoids in Bacteria, steroids in Eukarya—are used as a membrane reinforcers by cells. In his proposal, however, the central question on the origin of the differences in lipid composition between bacterial and eukaryal membranes by one hand—diacylgyceryl phospholipids—, and archaeal on the other—polyprenyl ethers (see, e.g.,19)—, remains unanswered. We need not only much more information on lipid composition and metabolism in Bacteria and Archaea but—as an essential data—the branching order of the three domains in the universal tree of life.

Were early cells either organic matter consumers or producers?

Oparin's suggestion was very clear. He assumed that early cells grew on preexisting organic matter of non-biological origin, i.e., the first metabolism was heterotrophic. That prebiotic organic matter might have originated from abiotic chemical syntheses in an early atmosphere or it might have been from extraterrestrial origin, having reached our planet by means of impacts of extraterrestrial bodies on the Earth. The origin of life in special chemical environments, such as deep sea vents, has also been suggested (37). However, there is also a scientific antagonistic vision confronted with Oparin's, which has been present for many years, even if it has been hardly taken into account in the biological literature. This idea—first mentioned by Haeckel—was developed by scientists such as the genetist H. J. Muller (29)—author of the hypothesis of the "living genes"—, C. R. Woese (44), F. M. Harold (11), H. Hartman (12) and, more recently, G. Wächtershäuser (41)—German patent's lawyer that published a new model of the origin of life in 1988 based on the use of the chemical energy of the anaerobic synthesis of pyrite as a metabolic fuel. All those authors' points of view are irreconcilable with Oparin's. In fact, they state that early living beings on Earth must have been producers (not consumers) of organic matter, i.e., the first metabolism was autotrophic. Mechanisms to capture the energy originated from inorganic chemical reactions or from visible light must have been at the basis of early metabolic activities. Nevertheless, the nature of the ancestral autotrophic pathway remains unknown, albeit some authors advocate in favor of an Archean version of the reductive tricarboxylic acid cycle (18,42). Also in this case an exhaustive and comparative study of the metabolic pathways in Bacteria and Archaea will shed light on this enigma.

Epilogue

The current knowledge allows an in-depth discussion of the enigma not yet solved about the origin of life. However, we are still far away from understanding it. Without Oparin's contribution, we would

never have reached the situation in which are now found. As Antonio Lazcano—who knew Oparin very well—pointed out (21), the best homage we can make to Oparin is to continue discussing his ideas and adding new doubts and mysteries to them.

Acknowledgments

A Catalan version of part of this paper was published in *Revista de Catalunya* in March 1994, exactly 100 years after Oparin's birth. Thanks are given to Mercè Piqueras for the translation into English. I also am indebted to Prof. Antonio Lazcano for his stimulating discussions on the origin of life during his stays in the University of València as a Visiting Professor. Thanks are given to DGICYT for financial support (grant no. PB92-0821).

References

1. Alberts, B. M. (1986). The function of the hereditary materials: biological catalyses reflect the cell's evolutionary history. Amer. Zool. **26**, 781–796.
2. Bartel, D., Szostak, J. W. (1993). Isolation of new ribozymes from a large pool of random sequences. Science **261**, 1411–1418.
3. Cairns-Smith, A. G. (1982). Genetic Takeover and the Mineral Origins of Life. Cambridge University Press, Cambridge, MA.
4. Chyba, C. F., Sagan, C. (1992). Endogenous production, exogenous delivery, and impact-shock synthesis of organic molecules: an inventory for the origins of life. Nature **335**, 125–132.
5. Crick, F. H. C. (1968). The origin of the genetic code. J. Mol. Biol. **38**, 367–379.
6. Deamer, D. W., Fleischaker, G. R. (1994). Origins of Life: The Central Concepts. Jones and Barlett, Boston, MA.
7. Ellington, A. D. (1995). Mundo del RNA y evolución in vitro. *In* Morán, F., Peretó, J. G., Moreno, A., (ed), Orígenes de la vida. En el centenario de A. I. Oparin. Ed. Complutense, Madrid, Spain.
8. Farley, J. (1977). The Spontaneous Generation Controversy from Descartes to Oparin. The Johns Hopkins University Press, Baltimore, MD.
9. Gilbert, W. (1986). The RNA World. Nature **319**, 618.
10. Haldane, J. B. S. (1929). The origin of life. The Rationalist Annual. Reprinted *in* Deamer, D., Flieschaker, G. (1994), Origins of Life: the Central Concepts. Jones and Barlett, Boston, MA.
11. Harold, F. M. (1986). The Vital Force: A Study of Bioenergetics, pp. 167–196. Freeman, New York, NY.
12. Hartman, H. (1975). Speculations on the origin and evolution of metabolism. J. Mol. Evol. **4**, 359–370.
13. Holm, N. (1992). Special issue: marine hydrothermal systems and the origin of life. Orig. Life Evol. Biosph. **22**, 1–242.
14. Joyce, G. F. (1989). RNA evolution and the origins of life. Nature **338**, 217–224.
15. Joyce, G. F. (1993). Climbing Drawin's ladder. Curr. Biol. **3**, 703–704.
16. Joyce, G. F., Orgel, L. E. (1993). Prospects for understanding the origin of the RNA world. *In* Gesteland, R. F., Atkins, J. F. (ed.), The RNA World, pp. 1–25. Cold Spring Harbor Lab. Press, Cold Spring Harbor, New York, NY.
17. Joyce, G. F., Schwartz, A. W., Miller, S. L., Orgel, L. E. (1987). The case for an ancestral genetic system involving simple analogues of the nucleotides. Proc. Natl. Acad. Sci. USA **84**, 4398–4402.

18. Kandler, O. (1994). The early diversification of life. *In* Bentston, S. (ed.), Early Life on Earth: Nobel Symposium No. 84, pp. 152–160. Columbia University Press, New York, NY.
19. Koga, Y., Nishihara, M., Morii, H., Akagawa-Matsushita, M. (1993). Ether polar lipids of methanogenic bacteria: structures, comparative aspects, and biosyntheses. Microbiol. Rev. **57**, 164–182.
20. Lazcano, A. (1986). Prebiotic evolution and the origin of cells. Treb. Soc. Cat. Biol. **39**, 73–103.
21. Lazcano, A. (1995). Alexander I. Oparin: apuntes para una biografía intelectual. *In* Morán, F., Peretó, J. G., Moreno, A., (ed), Orígenes de la vida. En el centenario de A. I. Oparin. Ed. Complutense, Madrid, Spain.
22. Lazcano, A., Guerrero, R., Margulis, L., Oró, J. (1988). The evolutionary transition from RNA to DNA in early cells. J. Mol. Evol. **27**, 283–290.
23. Lazcano, A, Miller, S. L. (1994). How long did it take for life to begin and evolve to cyanobacteria? J. Mol. Biol. **39**, 546–554.
24. Margulis, L., Sagan, D. (1986). Microcosmos. Four billion years of evolution from our microbial ancestors. Spanish version by Piqueras, M. (1995), Microcosmos. Tusquets, Barcelona, Spain.
25. McKay, C. P. (1991). Planetary evolution and the origin of life. Icarus **91**, 93–100.
26. Miller, S. L. (1953). Production of amino acids under possible primitive Earth conditions. Science **117**, 528–529.
27. Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature **191**, 144–148.
28. Morán, F., Peretó, J. G., Moreno, A., (ed) (1995). Orígenes de la vida. En el centenario de A. I. Oparin. Ed. Complutense, Madrid, Spain.
29. Muller, H. J. (1929). The gene as the basis of life. Proc. Int. Congr. Plant Sci. **1**, 897–921.
30. Noller, H. F., Hoffarth, V., Ziminiak, L. (1992). Unusual resistence of peptidyl transferase to protein extraction procedures. Science **256**, 1416–1419.
31. Oparin, A. I. (1924). Proiskhodenie Zhizni. Moscovky Rabotchii, Mockba English translation *in* Deamer, D., Flieschaker, G. (1994), Origins of Life: the Central Concepts. Jones and Barlett, Boston, MA.
32. Orgel, L. E. (1968). Evolution of the genetic apparatus. J. Mol. Biol. **38**, 381–393.
33. Orgel, L. E. (1992). Molecular replication. Nature **358**, 203–209.
34. Oró, J. (1961). Comets and the formation of biochemical compounds on the primitive Earth. Nature **190**, 389–390.
35. Oró, J. (1963). Studies in experimental organic cosmochemistry. Ann. N.Y. Acad. Sci. **108**, 464–481.
36. Ourisson, G., Nakatani, Y. (1994). The terpenoid theory of the origin of cellular life: the evolution of terpenoids to cholesterol. Chemistry & Biology **1**, 11–23.
37. Peretó, J. G. (1994). Orígenes de la evolución biológica. Eudema Biología, Eudema, S. A., Madrid, Spain.
38. Peretó, J. G. (1995). Origen de la célula. *In* Morán, F., Peretó, J. G., Moreno, A., (ed), Orígenes de la vida. En el centenario de A. I. Oparin. Ed. Complutense, Madrid, Spain.
39. Schopf, J. W. (1993). Microfossils of the Early Archean Apex Chert: new evidence of the antiquity of life. Science **260**, 640–646.
40. Sleep, N. H., Zahnle, K., Kasting, J. F., Morowitz, H. J. (1989). Annihilation of ecosystems by large asteroid impacts on the early Earth. Nature **342**, 139–142.
41. Wächtershäuser, G. (1988). Before enzymes and templates: theory of surface metabolism. Microbiol. Rev. **52**, 452–484.
42. Wächtershäuser, G. (1990). Evolution of the first metabolic cycles. Proc. Natl. Acad. Sci. USA **87**, 200–204.
43. Woese, C. R. (1967). The Genetic Code: the Molecular Basis for Genetic Expression. Harper & Row, New York, NY.
44. Woese, C. R. (1979). A proposal concerning the origin of life on the planet Earth. J. Mol. Evol. **13**, 95–101.
45. Woese, C. R., Kandler, O., Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. USA **87**, 4576–4579.

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Cyril Ponnamperuma (1923–1994)

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Cyril Ponnamperuma was a native of Sri Lanka (Ceylan) who moved to the United States in 1959, and in 1967 became a naturalized citizen. His early education was in Sri Lanka and India where he received a baccalaureate in philosophy. He then proceeded to London where he obtained a B. Sc. (Honors) degree in Chemistry at Birkbeck College, University of London, in 1959. During this time, he had the privilege of being associated with Professor J. D. Bernal, a pioneer in the field of the origin of life. After his studies at the University of London, Ponnamperuma attended the University of California, at Berkeley. I was also at Berkeley at that time and remember so well the day I met Cyril. He had just arrived at Berkeley, and joined our ranks as a new graduate student in Melvin Calvin's lab. During coffee hour around the big white conversation table, someone asked him what he would do for his doctoral thesis and how long he thought it would take him to complete it. Cyril said he wasn't quite sure exactly what the project would be, but he expected to finish in two years. He was characteristically optimistic and a ferociously hard worker. After two and a half years at Berkeley, he received his doctorate in Chemistry under the direction of Nobel Laureate Professor Melvin Calvin.

In 1962, he was awarded a National Academy of Sciences Resident Associateship, tenable with NASA at the Ames Research Center, Moffett Field, California. In 1963, he joined NASA's Exobiology Division and became Chief of the Chemical Evolution Branch. The primary goal of his laboratory was the study of the origin of life. When the Apollo program was established, he was selected as a principal investigator for organic analysis. He was closely involved with NASA in the Viking and Voyager programs. He served as a member of both SESAC (Space and Earth Sciences Advisory Council) and LSAC (Life Sciences Advisory Council) of NASA. Until his death, he was a member of the Governor's Advisory Council for Education for the State of Virginia.

Ponnamperuma was associated with many universities in the United States and abroad. He was on the visiting faculty of Stanford University, the University of Nijmegen in the Netherlands, and the Sorbonne. The Indian Atomic Energy Commission appointed him a Distinguished Visiting Professor in 1967. In 1970 and 1971, he was Director of the UNESCO Program for the Development of Basic Research in Sri Lanka. In 1982, the Chinese Academy of Sciences invited him to lecture in the People's Republic of China.

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After several years at Ames, California, he moved his Laboratory of Chemical Evolution to the University of Maryland, at College Park, where he and his work continued to occupy a central position in this field. Since July 1971 he was at the University of Maryland as Professor of Chemistry and Director of the Laboratory of Chemical Evolution. Among his teaching responsibilities were a graduate course on Chemical Evolution and an interdisciplinary undergraduate course on Life in the Universe. In 1976, he was a Phi Beta Kappa visiting scholar. In 1978, he was named a Distinguished Professor of the University of Maryland. In 1980, the International Society for the Study of the Origin of Life awarded him the first A. I. Oparin Gold Medal for the "best sustained program" on the origin of life. He determined to turn his indefatigable energy, organizational talents and great interpersonal skills building and helping others to build science in developing countries. While realizing the obvious importance of physical facilities for the conduct of science, with characteristic wisdom he realized that the development and nurturing of an intellectual infrastructure was the key. He saw that only if mechanisms existed that identified, trained and supported the right people there would be even the possibility for everything else to fall into place. This emphasis on the development of human resources was always the heart of all his many generous efforts to encourage the building of science in the developing world.

He was a D. SC. (Honoris Causa) of the University of Sri Lanka (1978), the University of Puget Sound (1982), the University of Peradeniya (1984), and the University of Sri Jayawardenepura (1985). In 1990, the President of Sri Lanka awarded him the "Vidya Jothi" (Luminary of Science) medal for his services to science and to Sri Lanka. In 1991, the government of France conferred on him the title of "Chevalier de Lettres et des Artes" for promoting international understanding. In 1991, the University of Maryland awarded him the first Distinguished International Science Award for a scholarly career combined with extraordinary services to the international community. In 1993, the Russian Academy of Creative Arts awarded him the first Harold Urey Prize for his outstanding contributions to the study of the origin of life.

The author of over four hundred publications related to chemical evolution and the origin of life Cyril Ponnamperuma occupied a central position in this field. He wrote and edited sixteen books on the subject, including «The Origins of Life» (Dutton, 1972), which has been translated into many languages. He was on the editorial board of the *Journal of Molecular Evolution* and for over a decade was editor-in-chief of the international journal *Origins of Life*.

In September 1994 he called me to outline his plans to collaborate with African colleagues in building a network of contacts that would foster a two-way exchange of people, ideas and resources between Africa and the industrialized North. The sensitive generosity of this effort was very characteristic of the way Ponnamperuma used to help people out. He spoke about the benefits that would accrue to SubSaharan Africa if the right sort of program was installed. Cyril understood, far better than most, just how much the "developed" world can receive when it gives to the "developing" world.

With his death the world of science has lost a creative, influential and articulate contributor. The large world of human needs and affairs has lost an effective, generous and humane benefactor. Those of us fortunate enough to have known him well, have lost a friend who took his personal commitments as seriously as his global ones.

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The beginnings of the International Society for the Study of the Origin of Life

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The question of the origin of life and its distribution in the universe has been in the mind of man for hundreds and probably thousands of years. In 1924 Alexander I. Oparin published a short book on the subject which was followed over the years by books in which he developed his original theme in much more detail. During this period, John B. S. Haldane had also published a book on this subject, although he and Oparin had never met nor were they aware of each other activity. In 1953 Stanley L. Miller, then a graduate student in Harold C. Urey's laboratory at the University of Chicago, did the first meaningful experiment related to the origin of life in which he synthesized amino acids in a flask containing an atmosphere of methane and water. Miller's experiments sparked a great deal of interest as well as laboratory activity over the next years.

In 1959, with the advent in the United States of a space program, which would allow us to begin to explore the universe and determine the uniqueness of life on Earth, there was an explosion of laboratory activity in the United States and countries around the world, including the Soviet Union, Japan, Germany, France, and Great Britain. In 1955, a symposium entitled «Modern View of Spontaneous Generation», was held at the Brooklyn Polytechnical Institute, in New York. Many scientists interested in the field attended that meeting, including George Wald, Urey and Miller. The first major meeting on the origin of life was a large convocation organized by Oparin in 1957 in Moscow named «The Origin of Life on the Earth». A book with the same title was published by Pergamon Press in 1959. Sydney Fox organized a second meeting in 1963 at Walkula Springs, Florida, entitled «The Origin of Prebiological Systems». This was supported by NASA through my position as Chief of the Exobiology Program. This also was a large meeting, including most of the people working in the field of the origin of life. It was at this meeting where Haldane and Oparin first met. As far as I know, it was the only time they actually met and discussed the question of the origin of life.

A few years later, in a meeting of the Radiation Society held in Cortina d'Ampezzo (Italy), Oparin, Fox and Cyril Ponnamperuma discussed forming an international society for the study of the origin of life. This was an important step in the thinking of the scientific community. On their return to the United States, Ponnamperuma and Fox approached me at NASA with the idea. This was followed by discussions between Fox, Ponnamperuma, Oparin and myself at the Ames Research Center (Moffett Field,

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California) and correspondence between all of us, especially between René Buvet in Franceand myself, until we prepared the necessary paperwork to initiate the organization of a new society for the study of the origin of life.

The year 1969 was a very active one in this context. We named pro tem officers with Oparin as President, Fox as Vice-President, Buvet as Treasurer, Ponnamperuma as Secretary and myself as Corresponding Secretary. While we were holding several meetings at Ames, I began to correspond with a number of scientists around the world soliciting their participation as members of an Executive Council. All of this was in preparation for the next meeting for the study of the origin of life, to be held at Pont-a-Mousson, France, starting on April 23, 1970. At this meeting an executive committee was elected for the society, and I was asked to begin drafting a constitution and by-laws so as to register the society as a non-profit organization in the state of Maryland in the U. S.

On September 20, 1971, a meeting on the «Origin of Life and Evolutionary Biochemistry» was held in Varna, Bulgaria, where a sufficient number of people was present to have further discussions and to clarify and review the constitution and by-laws that I had prepared by that time. On June 5, 1972, the International Society for the Study of the Origin of Life, ISSOL, was incorporated. The Board of Directors consisted of: A. I. Oparin, M. Florkin, S. W. Fox, R. Buvet, R. S. Young, S. Akabori, M. Calvin, K. Dose, G. Eglinton, A. Katchalsky, A. A. Krasnovsky, E. Kreps, F. Lipmann, S. L. Miller, C. Ponnamperuma, G. Porter and P. Sylvester-Bradley.

The first meeting of ISSOL as a scientific society was held in Barcelona, Spain, in 1973. Table 1 lists the ICOL–ISSOL meetings. The presidents of ISSOL have been: A. I. Oparin, USSR (1974–78), F. Egami, Japan (1978–1983), C. Ponnamperuma, USA (1983–1986), S. L. Miller, USA (1986–1989), J. Oró, USA (1989–1993) and J. Ferris, USA (1993–1996). The Society has been meeting approximately every three years and has been successful in producing and exchanging data relevant to the questions of the origin and early evolution of life.

TABLE 1. Meetings of ICOL–ISSOL*

Year	Location	ICOL	ISSOL	Registrants	Countries
1957	Moscow, USSR	1	–	n/a†	n/a
1963	Coral Gables, Florida, USA	2	–	n/a	n/a
1970	Pont-a-Mousson, France	3	–	n/a	n/a
1973	Barcelona, Spain	4	1	n/a	n/a
1977	Kyoto, Japan	5	2	~170	n/a
1980	Jerusalem, Israel	6	3	~120	~15
1983	Mainz, Germany	7	4	275	25
1986	Berkeley, California, USA	8	5	273	29
1989	Prague, Czechoslovakia	9	6	237	31
1993	Barcelona, Spain	10	7	406	23
1996	Orléans, France	11	8		

Table prepared by Sara Acevedo, NASA Ames Research Center.

* ISSOL was incorporated on June 5, 1972.

† n/a, not available.

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Book Reviews

Vital Dust

Life As a Cosmic Imperative

Christian de Duve

Basic Books., a Division of Harper Collins Pub. Inc., 1995. 362 pp. ISBN 0–465–09044–3.

In the preface of the book, the author confesses his early attraction towards the study of life. But because "active science narrows the mind more often that it broadens", we had to wait more than 50 years for this history of life that, as other critics have stated—and I agree with them—, only someone of the de Duve's stature and erudition could have written. But this lapse of time has not been lost time, neither for him, nor for us and for science. Christian de Duve belongs to that sort of open-minded scientists whose knowledge, interests and curiosity overpass their own field specialization, and in 1991 he gifted us with an advance of his going back to his old dream with *Blueprint for a cell.*

Christian de Duve was awarded the Nobel Prize for Medicine or Physiology in 1974, shared with Albert Claude and George Palade, for their discoveries on the structural and functional organization of the cell. Although the most widely known, the Nobel Prize has not been the only award he has received throughout an active life in research and teaching in Europe and USA.

Vital Dust is a journey from the beginning of the past (3500 millions years ago) to the end of the future (in 5000 millions years after present). For this journey, the author provides the travelers with the complete baggage to enter into the simple and complex processes of the origin and evolution of life. Basically, this baggage consists of concepts such as *the Unity of life*, refering to the fact that all extant living organisms evolved from a single common ancestor, which emerged about 3800 years ago. *The tree of life,* built up mainly from the traces left in the fossil record, and from discoveries on inner structure, organization and composition of macromolecules. *The antiquity of life*, which provides the reader with information on the time scale of the tree of life. It takes into account the paleontological trees (fossil traces), the molecular trees (the tolerable mutation rate in the course of evolution) and the bacterial fossils (stromatolites), which are the most recent contribution to the study of the origin and evolution of life. *The cradle of life* is the point at which the author mentions the different theories about where life originated, either on Earth or from space? Anyway,—as the author states—, what really matters is how life originated, whatever the place where this phenomenon took place. *The probability of life* deals with a set of theories, controversies, religious and philosophical concepts arisen from queries on the origin of life that science has not yet been able to solve. With this equipment, the author simply wishes to examine the scientific validity of the probability argument that could consider of impeccable logic: if life would be the result of a single event. But the consideration of the high complexity of a living

cell alters the probability assessment. As a scientist, de Duve chose to provide a summary of available evidences and to share his personal interpretation.

In its thirty-one chapters, *Vital Dust* offers the most up-dated text on the origin of life. The chapters are distributed in seven parts, each one representing a different level—age—of the complexity of life. Forty-eight pages of notes—with useful comments on events and literature—, plus a glossary and additional reading, complete this book indispensable for scientists, students and everybody interested in the origin, evolution and nature of life, as well as on the controversies, theories and approaches to those matters.

The author makes it clear his own position and creeds about two central points, one of them being the origins of life, which "is increasingly explained strictly in terms of the laws of physics and chemistry", and the other one about the place where life originated. "I shall, therefore, assume that life was born right where it actually is: here on Earth", states de Duve. Books like this convert the adventure of life in a personal adventure, in which the reader feels compelled to actively participate.

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Early Life on Earth

Nobel Symposium No. 84

Stefan Bengtson (ed.)

Columbia University Press, New York, 1994. 630 pp. ISBN 0-231-08088-3.

This book gathers the contributions presented to the Nobel Symposium which took place at the Nobel's Björkborn Foundation—where Alfred Nobel had his home and laboratory—in Karlskoga (Sweden) in May 1992. Geologists, paleontologists, evolutionary biologists, molecular biologists, biochemists, etc, from twelve countries participated in that meeting presenting a wide array of hypotheses and opinions on the origin and early evolution of life on earth. The reader will not find in this book a continous, coherent tale of the history of life on our planet. What the book offers us are the visions that different researchers have of this history, sometimes conflicting among them

The book is divided in four "Themes", preceded by a preface and by an introduction by Stephen Jay Gould. Although Gould confesses to be an ignoramus in many of the scientific fields involved in the symposium, he shows an amazing scholarly, and stirs up the reader's curiosity and interest in the mystery that the following chapters of the book can unravel. As Gould himself states, the book, despite the lack of cohesion which can be expected from such an interdisciplinary meeting, has a common thread: the history of life on earth. The researchers who met in Karlskoga to participate in this Nobel Symposium played the role of historians, their expertise having been applied to unravel the obscure, unknown period that preceded the last almost ten per cent of the earth's history.

The first theme, under the title "Life's Gestation and Infancy" covers two different periods. It deals first with the changes undertaken by our

planet since its inception, with all the phenomena that led to the first living organism; and the evolution of those primordial cells. That period is full of mystery, open to any kind of speculation. Secondly, it deals with a period of which we have a fingerprint in the fossil record. The first two chapters—"The planetary setting of prebiotic evolution", by S. Chang, and "Early environments: Constraints and opportunities for early evolution", by D. R. Lowe,—are devoted to study the geological, chemical characteristics of the early earth. Nine chapters follow that are crucial to understand—or, even better, to imagine—the phenomena which might have occurred to eventually produce a living being, which means an entity capable of transforming energy and self-reproducing. J. Oró describes what is currently known about the chemical stages which led to the first living being. A. Lazcano offers his plausible hypotheses on both the transition from nonliving to living and the role of RNA at the first steps of life. The chapters written by K. O. Stetter, O. Kandler, B. K. Pierson and M. L. Sogin show how the knowledge of current microorganisms can help us to understand the beginning of biological diversity and the evolution of the first ecosystems on earth.

The second theme, "The Maturation of Earth and Life", includes the period spanning from the Archaean to the Proterozoic, during which dramatic changes in the composition of the earth's atmosphere took place. The formation and stabilization of the continents changed the planet's surface, which is a likely explanation for the proterozoic evolution. The fossil record of that period is abundant and diverse. Although prokaryotes were still predominant in the biota, eukariotes had already emerged, and their evolution can be studied and understood from the study of extant organisms. M. R. Walter describes the role of stromatolites as geological forces, and how the study of their evolutionary history can be of help to understand the evolution of the organisms involved in their construction. G. Vidal tries to elucidate the nature of marine ecosystems at the end of the Proterozoic by studying the abundant mineralized remains from that period. L. Margulis and J. E. Cohen discuss the significance of symbiosis in the evolutionary path which led to eukaryotes.

The third and last theme "Multicellularity and the Phanerozoic revolution" presents different points of view and ideas to explain the enormous animal diversification that took place in the limit between the Proterozoic and the Phanerozoic. The authors' contributions provide a big profusion of data to explain their different various hypotheses on the Cambrian explosion, which range from environmental changes (A. Knowll) and key biological innovations (Bergström) to ecological interactions (S. Bengston) and genetic control of development (J. Valentine).

The book reflects the enormous impact of molecular biology in the study of early evolution. The comparison of nucleic acid sequences from different organisms has made it possible to produce new genealogic trees and to obtain "molecular clocks", which indicate where some given changes in the DNA sequences of certain genes may have taken place. It is indeed amazing the new light shed on phylogenetic studies by the latest advancements in molecular biology.

However, and despite those advancements, the book makes it clear the mysteries still pending to be solved in the history of life on earth. As a matter of fact, at no moment does the book intend to offer an answer to those mysteries. But it is a step towards their explanation.

Mercè Piqueras
Microbiología SEM

What is Life?

Lynn Margulis, Dorion Sagan

Simon & Schuster, New York, 1995. 208 pp.
ISBN 0-684-81326-2

This book is a fresh look at this most basic, but mostly unaddressed, question of biology. At first the question of "what is life?" might seem to belong to that group of questions for which we simply cannot imagine an answer as with the question "what does it mean to be eternal?" It is an area where science runs out of answers and gives way, by default, to myth and religion. But Margulis and Sagan take a different path. While devoting two chapters to the origin of life and the basic criteria of living organisms, they use the bulk of the book to explain how the different forms of life are complex variations and complications on the basic attributes of life.

Although a popular book for the general reading public, written in an easy-to-read style, it is a very intellectual one that is the product of years of scholarly work in a wide range of fields, a synthesis that Margulis and Sagan have drawn from experts and their own unique reading of life. As Niles Eldredge writes in the Foreword, in this book "we encounter ways of thinking about life that could not possibly arise from simple introspection."

The book consists of nine chapters starting with the concept of self maintenance, or autopoiesis. This is followed by a review of philosophical views of life and death, including those of Giordano Bruno, Descartes, Spinoza, Humboldt, Darwin and Vernadsky. The origin of life takes up the next chapter, in which the authors agree with Harold Morowitz that metabolism evolved after membrane-bound cells arose. We are then treated to a chapter each on the five kingdoms of life: bacteria, protoctists, animals, fungi and plants, dealing with many of the aspects of the evolution of these organisms that Margulis has made famous. The authors clearly have a very microbiologically-oriented view of life, as indicated in the title of the chapter on bacteria, Masters of the Biosphere. A final chapter entitled Sentient Symphony raises the prospect of bacterial "choice" and "memory", and suggests that the learned habit of one generation can be transmitted to the physiology of the next. And much more.

One might ask, among other things, isn't the five kingdom scheme archaic in light of the widespread use of the three domain scheme of archaeobacteria, eubacteria and eukaryota. Margulis' response would likely be that the symbiogenetic origin of protists, plants, animals and fungi are more important than the distinctions between two groups of bacteria. In other words, there are two reasonably adequate ways of describing the same thing, something science, in its overly reductive way, has a hard time accepting. Furthermore, the symbiogenetic theory of cell evolution, although widely accepted, still goes unmentioned in many works such as the 1992 book edited by J. William Schopf, *Major Events in the History of Life*.

The book has a handsome design with seventy full color photographs (disclaimer: I worked for the authors in collecting the photographs two years ago). There are several charts and graphs having the distinctive Margulis/Sagan signature: the human-centered timeline (1 page) next to the true time scale (10 pages) or the chart of the biominerals produced throughout the five kingdoms headed, "Contrary to popular belief minerals and animals do not belong to separate kingdoms." *What Is Life?* is an enjoyable read, a hearty product of the Margulis/Sagan wisdom, that will stimulate readers for many years.

Michael Dolan
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Les origines de la vie

Marie-Christine Maurel

Syros (Pub), Paris, 1994. 208 pp. ISBN 2-84146023-1.

Sometimes a popular science book arrives to our hands that actually deserves the epigraph of the publisher's series. This is the case with *Les origines de la vie* by Marie-Christine Maurel recently published by Syros (pub) in the series "Comprendre". In a clear, easy style, not exempt from both scientific and epistemological rigor, Maurel addresses some of the most fascinating questions facing humankind: our understanding of both the origin of life and the origin of the Universe itself.

This unified way to view the origins and evolution of the Universe—including life on Earth—was already adopted in *The Universe and Life* by G. S. Kuttler. In that remarkable text book, the author used a chronological approach in the order of presentation of the topics. Similarly, M.-C. Maurel also follows the logical progression of "physical evolution" providing the framework for the "biological evolution". Both concepts are considered here in terms of physical and chemical laws.

The development of the expanding Universe is presented here as the physical evolution of a closed system of atomic particles and quantic fields. Therefore, on an universal scale, the four forces of Nature (gravity, electromagnetic, strong nuclear and weak nuclear) are constrained to a dissipate complexity and spread dissorder. In contrast, the open character of living systems allowed the biological evolution based on mechanisms of molecular auto-organization of increasingly complexity, on the existence of genetic information, and on the changes this information undergoes with time. Though, in the early molecular aggregates, the localized pressures of mutational variation and natural selection led to the origin of self-maintainig systems: the first organisms on Earth.

Concisely written, this book is intented for people curious about the surrounding world and interested in having a comprehensive view at our evolutionary heritage, without necessarily possesing the technical background of the specialist. But also to those students who want to acquaint themselves with some of the scientific issues beyond their immediate areas of concern. It tells the story of the origins and evolution of both the Universe and life on Earth, emphasizing biological evolution. As an introduction to the topic, this book not only provides with essential background information, but also describes new molecular approaches to the study of microbial evolution.

The presentation is didactic, attractive and enjoyable for the reader. References to the original articles, marginal notes, figures and diagrams are constant through the text. Furthermore, each chapter contains numerous comments and short essays that are boxed to set them off from the main text. They offer additional information about specific subjects, such as the solar system, dating of fossils, the SETI (Search for Extra-Terrestrial Intelligence) research program, archaeobacteria, proto-oncogens, clays and enzymatic activity, introns, editing, the PCR technique, symbiosis, molecular biology and gene sequencing, etcetera. Finally, to help the reader, the author assembles a list of specific words and succint definitions in a glossary at the end of the book. The books and articles cited are the essential references to the topics, even though the most recent and relevant publications are not included. This is probably due to the condition of popular science book.

The book is divided into nine chapters, beginning with an overview of the historical development of science, the theories, methods,

evidences and upheavals of our current knowledge of the origins and evolution of life. Following the opening chapter, Maurel delineates the likely events of early "Big-Bang" cosmology and the necessary time frame for galactic and solar system development, with particular attention to those events that were most crucial for the eventual genesis of life on Earth.

Next, the author focuses on the beginning of the prebiotic chemistry and the experimental models for the study of the abiological molecular synthesis, the early energy-generating systems and self-replicating mechanisms, required for setting the stage of early life and further evolution. The major stages of biological evolution are described through the increase in metabolic and reproductive complexity and the development and diversification of cellular organisms. Special emphasis is laid on such topics as the so-called "RNA-world" or the era of "RNA life", the evolution of the genome and the origin and evolution of the eukaryotic cells according to L. Margulis serial endosymbiotic theory.

Finally, some of the recent developments in molecualr biology or still-in-progress experiments are also included, as well as new interpretations of the fossil records, which are gradually altering and adding to the synthetic theory of evolution.

Even if this book does not cover all possible recent theories about biological evolution, it provides with a general an essential background of knowledge, and stimulates to delve further into these exciting questions.

Perhaps with the final synergistic theory of the origines of life on our "pale wandering firefly" traveling through time and space, one day we could say "... oh butterflies, I have learnt more from you than from the books of all the Brahamans" (Buddha).

Carmina Rodríguez-Fernández
Universidad Complutense de Madrid

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Some books on the origin or early evolution of life

ARNAU, C., CARBÓ, R. (1973). El origen de la vida. Biblioteca Salvat de Grandes Temas. Salvat Editores, S.A., Barcelona. 144 pp. *(With a long interview to Joan Oró. In Spanish.)*

BENGTSON, S. (ed.) (1994). Early Life on Earth. Nobel Symposium No. 84. Columbia University Press, New York, NY. 598 pp. *(See review in this issue.)*

BRACK, A., RAULIN, F. (1991). L'évolution chimique et les origines de la vie. Masson, Paris. *(In French.)*

BRODA, E. (1975). The Evolution of the Bioenergetic Processes. Pergamon Press, Oxford.

CARLE, G. C., SCHWARTZ, D. E., HUNTINGTON, J. L. (1988). Exobiology in Solar System Exploration. U.S. Government Printing Office, NASA, Washington. 298 pp.

DEAMER, D. W., FLEISCHAKER, G. R. (ed.) (1994). Origins of Life. The Central Concepts. Jones and Bartlett Pub., Boston, MA. 418 pp.

DE DUVE, C. (1990). Blueprint for a Cell. The Nature and Origin of Life. Neil Patterson Pub., Burlington, NC. 276 pp.

DE DUVE, C. (1995). Vital Dust. Life as a Cosmic Imperative. BasicBooks, New York, NY. 362 pp. *(See review in this issue.)*

DYSON, F. (1985). Origins of Life. Cambridge University Press, Cambridge, United Kingdom. 82 pp.

EIGEN, M. (1992). Steps towards Life. A Perspective on Evolution. Oxford University Press, New York, NY. 168 pp.

GARCÍA MOLINA, V., AGUILERA, J. A. (1985). ... y la Tierra palpitó. El origen de la vida. Ciencias de la Naturaleza, Hermann Blume, Madrid. 230 pp. *(An original discussion on the origin of metabolism. In Spanish.)*

GINER-SOROLLA, A. (1983). Un nou gènesi: A l'entorn dels orígens. Edicions 62, S.A., Barcelona. 192 pp. *(In Catalan.)*

HARTMAN, H., LAWLESS, J. G., MORRISON, P. (ed.) (1985). Search for the Universal Ancestors, the Origin of Life. U.S. Government Printing Office, NASA, Washington. 130 pp.

LAZCANO-ARAUJO, A. (1983). El origen de la vida. Evolución química y evolución biológica. Editorial Trillas, S.A., México, D.F. 108 pp. *(In Spanish.)*

LAZCANO, A. (1992). La chispa de la vida. Pangea Eds., México, D.F. 142 pp. *(In Spanish.)*

LOCQUIN, M. V. (ed.) (1987). Aux origines de la vie. Fayard, Paris. 360 pp. *(In French.)*

MARGULIS, L. (1988). Early Life. Jones and Bartlett Publ., Boston, MA. 140 pp.

MARGULIS, L., SAGAN, D. (1995). What is Life? Simon & Schuster, Inc., New York, NY. 208 pp. *(See review in this issue.)*

MAUREL, M.-C. (1994). Les origines de la vie. Syros, Paris. 208 pp. *(See review in this issue. In French.)*

MASON, S. F. (1992). Chemical Evolution. Origin of the Elements, Molecules, and Living Systems. Oxford University Press, New York, NY. 302 pp.

MOROWITZ, H. J. (1992). Beginnings of Cellular Life. Metabolism Recapitulates Biogenesis. Yale University Press, New Haven, CT. 186 pp.

PERETÓ, J. G. (1994). Orígenes de la evolución biológica. Eudema, S.A., Madrid. 96 pp. *(In Spanish.)*

PI SUÑER, A. (1941). Principio y término de la biología. Ministerio de Educación, República de Venezuela, Caracas. 376 pp. *(Commemorative edition published in 1981. A rare, interesting book hardly known. In Spanish.)*

THUAN, T. X. (1988). La mélodie secrète. Fayard. Paris. 390 pp. *(In French.)*

SCHOPF, J. W. (ed.) (1983). Earth's Earliest Biosphere. Its Origin and Evolution. Princeton University Press, Princeton, NJ. 544 pp.

SCHOPF, J. W. (1992). Major Events in the History of Life. Jones and Bartlett Pub., Boston, MA. 184 pp.

SCHOPF, J. W., KLEIN, C. (ed.) (1992). The Proterozoic Biosphere. A Multidisciplinary Study. Cambridge University Press, New York, NY. 1348 pp.

Normas para los autores

Microbiología SEM (la revista científica de la Sociedad Española de Microbiología, SEM) acepta artículos y notas de investigación originales dentro del campo de la microbiología y, ocasionalmente, artículos de revisión. Textos en inglés (preferentemente) o español. La aceptación corresponde al Consejo Editorial. Sólo se admitirán trabajos inéditos que no estén pendientes de publicación en cualquier otra revista. Los originales publicados en *Microbiología SEM* podrán ser reproducidos siempre que se indique su origen.

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Seeberg, E., Nissez-Meyer, J., Strike, P. (1976). *den*V gene of bacteriophage T4 determines a DNA glycosilate specific for pyrimidine dimers in DNA. J. Virol. **35**, 790–797.

Tomasz, A. (1984). Building and breaking in the cell wall of bacteria. The role for autolysins. *In* Nombela, C. (ed.), Microbial Cell Wall Synthesis and Autolysis, pp. 3–12. Elsevier Science Pub., Amsterdam, Netherlands.

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