

**ABSTRACTS OF THE ORAL PRESENTATIONS FROM THE
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PLENARY AND INVITED LECTURES

THE RNA WORLD AND THE ORIGIN OF LIFE

Leslie Orgel

The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

Recent X-ray studies of ribosomal structure provide strong evidence that there was once an RNA world in which RNAs functioned both as genetic and catalytic molecules without assistance from RNA-coded peptides or proteins. This simplifies the problem of the origin of life in some ways because it is now only necessary to explain the origin of RNA, but it makes inference from biochemistry to prebiotic chemistry more problematical because all biochemicals except RNA may have been invented by RNA. I will first review recent experimental studies that relate to the possible *de novo* origin of the RNA world. Then I will emphasize attempts to develop replicating systems different from and simpler than RNA, that might have acted as midwives for the birth of the RNA world.

FORMATION AND EVOLUTION OF ORGANICS IN SPACE

Pascale Ehrenfreund

Leiden Observatory, P.O. Box 9513, 2300 RA Leiden, The Netherlands

Life, as we know it, is based on carbon. In the early Universe only light elements, such as H and He (and traces of other light elements) were formed. The formation of heavier elements had to await the formation of stars. Nucleosynthesis of heavy elements in stars, such as carbon, allowed the formation of organic molecules, which are currently widespread in our Galaxy and beyond. Today biogenic elements (H, C, N, O, S, P) and organic matter are some of the major constituents of the Universe. The step from atoms to molecules begins with the expulsion of nucleosynthetic products into the interstellar medium (ISM) by stellar winds and supernova explosions. Cosmic matter is predominantly formed in dense molecular clouds, where molecular synthesis takes place in the gas or solid phase or in the extended atmospheres of evolved stars that slowly lose mass. The only known life in the Universe resides on a planet orbiting a G-type star. Stars like the Sun are born in dense molecular clouds, and these also provide the initial organic inventory available to protostellar disks for the formation of planets. Analysis of cosmic matter – be they gaseous or solid – remains a vital tool for monitoring their evolution in space and tracing their path from star-forming regions to planetesimals and Solar System bodies, such as planets, comets and asteroids. Remnant planetesimals and their fragments, such as interplanetary dust and meteorites, delivered material to the early planets. Part of this material included organic matter, which may have been the crucial raw material triggering an increase in the complexity of chemical reactions that ultimately led to the origin of life.

PREBIOTIC CHEMISTRY, CATALYSIS AND RNA SYNTHESIS

James P. Ferris

Department of Chemistry and the New York Center for Studies on the Origins of Life, Rensselaer Polytechnic Institute, Troy, N. Y. 12180, USA

The starting materials for prebiotic chemistry on Earth probably were the organics delivered to the primitive Earth on the dust and meteorites generated in the asteroid belt. Comets contain organics and may have also been a source of the prebiotic starting materials. Chemical processes on the primitive Earth may have converted some of these organics to nucleotides, the building blocks of RNA. Activated nucleotides do not condense to form RNA in aqueous solution so mineral and metal ion catalysts were required to generate the biopolymers that initiated the first life on Earth. Montmorillonite clay catalyzes the conversion of activated mononucleotides to RNA. Catalysis can limit the number of reaction pathways so that selected sequences of long biopolymers form rather than all possible isomers of short oligomers.

THE TNA-FAMILY OF NUCLEIC ACID SYSTEMS: PROPERTIES AND PROSPECTS

Albert Eschenmoser

Laboratory of Organic Chemistry, Swiss Federal Institute of Technology, Hönggerberg, HCI-H309, CH-8093 Zürich, Switzerland and The Skaggs Institute for Chemical Biology at the Scripps Research Institute, MB16, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

The (L)-alpha-threofuranosyl-(3'-2')-oligonucleotide system ('TNA') is a structural and functional analog of RNA, containing the four-carbon sugar L-threose in place of D-ribose. TNA is the structurally simplest potentially natural nucleic acid alternative derived thus far from RNA's structural neighborhood and 'speaks', in contrast to most of the previously encountered alternatives, the same 'base-pairing language' as do RNA and DNA.

The lecture will summarize what we know about the structure and the properties of TNA and will then discuss the results of recent investigations that have been inspired by the apparent simplicity of TNA's molecular architecture.

ALTERNATIVE PARADIGMS FOR THE RNA-CATALYZED REPLICATION OF RNA

Gerald F. Joyce, Kathleen E. McGinness, and Natasha Paul

The Scripps Research Institute, 10550 N. Torrey Pines Road La Jolla, CA 92037, USA

The ability of RNA to catalyze the replication of RNA molecules is an activity that would have been necessary for RNA-based evolution in the RNA world. In addition to exploring the 'standard model' of template-directed polymerization of RNA, we have investigated alternative paradigms involving either autocatalytic or propagative systems that rely on RNA catalysis. Our aim is to broaden the view of

how RNA-based evolving systems might operate, recognizing that replication as it occurs in contemporary biological systems is not necessarily the best model for replication during the early history of life on Earth.

Pursuing the standard model, the hc ligase ribozyme, which catalyzes the template-directed joining of oligonucleotides, was evolved to operate on a separate double-stranded RNA substrate. One strand of the substrate acts as a template and the other consists of two or more pieces of RNA that are joined by the ribozyme. The ribozyme exhibits a high level of sequence generality in this reaction and can catalyze the template-directed addition of NTPs onto the 3' end of an oligonucleotide primer.

A second approach to RNA-catalyzed RNA replication involves autocatalytic systems in which the ribozyme catalyzes the assembly of copies of itself from component oligonucleotides. Such a system was constructed from the R3C ligase ribozyme, restructuring it as a symmetric homodimer, with each monomeric unit containing a catalytic center that is formed by the covalent joining of two oligonucleotides. The ribozyme catalyzes the joining reaction, resulting in an exponential increase in the number of ribozyme molecules over time. A third approach involves propagative systems in which the ribozyme expands through a mechanism akin to crystal growth. The R3C ligase was divided into four component oligonucleotides that can associate non-covalently to form a functional catalyst. The component oligonucleotides can assemble into a three-dimensional lattice with a large variety of possible branching patterns. Some of those patterns form more readily than others and are more amenable to catalysis, providing the basis for Darwinian selection to enrich the most favorable branching patterns.

GENOME SCALE ANALYSES AND THE EARLY EVOLUTION OF LIFE

Johann Peter Gogarten

*Dept. of Molecular and Cell Biology, Univ. of Connecticut, Storrs, CT 06269, USA
(gogarten@uconn.edu)*

Darwin's work on the origin of species and the neo-Darwinian synthesis suggested that over long periods of time evolution could be described as a steadily bifurcating tree. The introduction of ribosomal RNA as a tool in microbial taxonomy by Carl Woese and George Fox let many microbiologist to assume that the concepts of animal and plant taxonomy could be extended to the realm of prokaryotes. The metaphor for organismal evolution that underlies a natural taxonomic system is a steadily bifurcating species tree, also known as the tree of life. A decade ago ribosomal RNA promised that one day it might be possible to place every extant organism on a universal tree of life, and the hope was that more genomic sequences would make this placement more accurate. However, the analyses of completely sequenced genomes initiated a reassessment of concepts in microbial evolution. While some molecular markers were found to agree with one another, others do not. Transfer of genetic information between divergent organisms has turned the tree of

life into a net or web, and genomes into mosaics. Different parts of genomes have different histories, and representing the history of genome evolution as a single tree appears inconsistent with the data. However, the exchange of genetic information is not so rampant as to have obliterated all phylogenetic signals. Horizontal gene transfer is not random, neither with respect to donors and recipients nor with respect to the type of genes that are transferred.

The talk will discuss approaches to study genome evolution; it will focus on the relationships between prokaryotic phyla, and will attempt a critical assessment of attempts to calibrate the early molecular record with respect to time.

GENOMIC INSIGHTS TO CYANOBACTERIAL EVOLUTION

George E. Fox¹, Janet L. Siefert², Kirt Martin¹, Yue Lu¹, Tom McNeil¹ and Shyla Yerrapragada¹

¹ Dept. Biology & Biochemistry, University of Houston, Houston, TX 77204-5001, USA; ² Dept. Statistics, Rice University, Houston, TX 77251-1892, USA

Complete genome sequence data is now available for six cyanobacteria representing a major portion of the diversity in this lineage. A comparative study of these genomes has been undertaken. In order to effectively display the results of such comparisons a software system known as the Genome Display Tool was developed. This system allows the user to readily visualize the distribution of various features within a whole genome context. The comparison allowed us to identify a core set of cyanobacterial genes that are uniquely found in the cyanobacterial lineage and therefore strong signatures of it. Surprisingly, the function of these signature genes is largely unknown suggesting that cyanobacteria may have long standing characteristic phenotypic features that are not well understood. These unidentified genes may effect how we interpret the ecological significance of cyanobacteria on the early Earth and it will therefore be important to determine their biochemical role. Our results also confirm earlier studies that showed that only a very small number of genes are uniquely shared between the various types of photosynthetic bacteria. The *Synechocystis* 6803 genome has been claimed to contain evidence of unusually large amounts of recent lateral transfer. An examination of the predicted laterally transferred genes shows there is remarkably little agreement about this.

ISOTOPIC EVOLUTION OF THE BIOGEOCHEMICAL CARBON CYCLE DURING THE PRECAMBRIAN

David J. Des Marais

Space Science Division, Ames Research Center, Moffett Field, CA 94035-1000, U.S.A.

Interactions between Earth and the biosphere were crucial for early biological evolution, and some of these interactions were reflected at least in part by stable carbon isotopic patterns preserved in Precambrian sedimentary rocks. Important long-term trends (e.g., solar luminosity increase, decline in size and rate of impacts,

decline in radiogenic heat flow, and the stabilization of continental masses) perhaps determined the timing and nature of the origins and early evolution of life. The key reservoirs of carbon reside in the atmosphere, ocean, crustal sediments and metasediments, and the mantle. Carbonates and reduced organic carbon constitute the two major crustal carbon reservoirs, and their markedly different isotopic compositions are a legacy of biological isotopic discrimination during the biosynthesis and subsequent utilization of organic carbon. Time-related changes in isotopic patterns within these two reservoirs indicate changes in biological isotopic discrimination, as well as changes in the relative rates of burial of organic versus carbonate carbon. The range of carbon isotopic compositions of sedimentary kerogens in rocks older than about 2.1 billion years is substantially larger than in younger rocks. Anaerobic microorganisms utilize a variety of pathways for CO₂ assimilation that vary substantially in their isotopic discrimination. This age-related decrease in the range of carbon isotopic compositions of sedimentary kerogens perhaps reflects the transition from a world where diverse populations of anaerobes contributed substantially to sediments to a world where primary production was dominated by cyanobacteria and eukaryotic algae. The development of oxygenic photosynthesis increased global productivity by some 100-fold, leading to enhanced inventories of fresh organic carbon. The fermentation of this organic matter produced hydrogen that, prior to the rise of an atmospheric oxygen inventory, might have greatly increased the rate of hydrogen escape to space. Hydrogen escape hastened the rate of oxidation of the surface environment. The onset after 2.45 billion years ago of major isotopic excursions for sedimentary carbonates indicates that a large fraction of sedimenting carbon was episodically buried as organic carbon. Such isotopic excursions confirm the global importance of oxygenic photosynthesis by 2.45 billion years ago. Such excursions were perhaps triggered in part by tectonic controls upon the burial and long-term preservation of sedimentary organic carbon. Such organic burial events hastened the development of a more oxidized surface environment that included increased abundances of sedimentary ferric iron and sulfates, as well as higher concentrations of seawater sulfate and atmospheric oxygen. Increases in the range of sulfur isotopic compositions of sedimentary sulfides and sulfates are consistent with a rise in seawater sulfate concentrations; and these increases coincided in time with the increases in rates of organic burial and in atmospheric oxygen levels. The isotopic patterns and trends observed for Precambrian sedimentary carbon and sulfur reservoirs offer additional insights into the nature and timing of the long-term evolution of the biosphere.

ASTROBIOLOGY: A PLANETARY PHENOMENON

Michael Meyer

Code SE, Solar System Exploration Division, NASA Headquarters, Washington, DC, 20546 USA.

Life may be a natural consequence of planet formation and so the study of the origin, evolution, distribution and future of life may be largely a planetary endeavor.

Life on our planet is the only known example in the universe and so we are relegated to this planet for the study of life. Astrobiology has adopted several approaches to study life on Earth and for determining the likelihood of life elsewhere. The first approach has been the Exobiology Program, centered around understanding the origins of life and which supports individual investigator research. Second has been the construction of consortia-type research in which researchers from different disciplines focus on a larger problem. This structure began with NASA Specialized Centers of Research and Training and has grown to include the Astrobiology Institute – a collection of competitively selected groups of researchers attacking problems in Astrobiology as individual teams and as a consolidated Institute. With the formation of an intellectual basis for exploring for life elsewhere, Astrobiology has initiated the competitive research and development program in instrument development (Astrobiology Science and Technology for Instrument Development [ASTID] Program) that would enable future mission instruments for the exploration of planetary bodies in the search for prebiotic chemistry, habitable environments (past or present), biomarkers, and possibly life itself. However, the act of exploring requires robust instrumentation, mobile robotic platforms, efficient operations and a high level of autonomy. To this end, Astrobiology has started a new research activity that promotes scientifically-driven robotic exploration of extreme environments on Earth that are analogous to suspected habitable environments on other planetary bodies. The program is called Astrobiology Science & Technology for Exploring Planets (ASTEP). Through this program, we will expand the known limits to life on Earth, develop methodologies for detecting biomarkers, and learn how to explore a rugged, novel environment while meeting astrobiology objectives. Perhaps Astrobiology is a natural consequence of planet formation, for what is more natural than for life to study life itself.

TITAN'S EXOBIOTICAL ATMOSPHERE: RECENT REVELATIONS

Athena Coustenis

LESIA, Paris-Meudon Observatory, 92195 Meudon Cedex, France

Titan, Saturn's largest satellite, is currently the only confirmed exobiological environment in our Solar System, bearing strong similarities to our own planet. Indeed, Titan's atmosphere is composed of nitrogen, methane and hydrogen essentially. The combination of these mother molecules produces an exciting organic chemistry in Titan's atmosphere, with traces of hydrocarbons and nitriles.

As a difference with our own planet we note the absence of significant amounts of oxygen (only traces of CO, CO₂ and more recently, H₂O have been discovered), as well as the low temperatures prevailing (180 K in the atmosphere and 94 K on the surface) that delay chemical reactions.

Still, Titan's uncanny resemblance to our own planet has motivated generations of scientists to studying it from both the space and from the ground, and has

initiated the Saturn-bound *Cassini/Huygens* ESA/NASA mission (arrival at Titan is scheduled for 2004).

Titan has been observed recently from the ground with adaptive optics systems which allow us to resolve its disk. In the acquired images we have found evidence for meteorological phenomena (such as haze variations like a morning fog). The presence of tropospheric clouds has been suggested from ground-based spectroscopy.

ISO, the infrared observatory has been also used to acquire high-resolution spectra of Titan which allowed us to find new components in its atmosphere, thus further constraining evolutionary and photochemical models.

I will present the implications of recent (ISO, ground-based) and future (Cassini) observations on the nature of Titan.

PUBLIC LECTURE:**ALFONSO L. HERRERA Y LA PLASMOGENIA: UNA TEORIA MEXICANA SOBRE EL ORIGEN DE LA VIDA**Lucía Perezgasga¹ y Erwin Silva²

¹ Instituto de Biotecnología, UNAM. Av. Universidad #2001 Col. Chamilpa. Apdo. Postal 510-3, Cuernavaca, Mor 62250, México; ² Facultad de Ciencias, UNAM. Circuito exterior, Cd. Universitaria, Apdo. Postal 70-407, México, D.F. 04510

In the second half of 19th century the natural sciences, particularly those related to the origins of life, faced a profound crisis. Theories as Panspermia, autocatalytic and heterocatalytic gene (Muller), or enzyme (Troland) theories, could not answer the question of the origins of life. On the other hand, Lamarck's and Darwin's evolutionary theories described the gradual transition from the inorganic to the organic world. Darwin's ideas were further developed by Haeckel, who thought that the first living forms were composed by cellular plasma. Herrera approached this problem experimentally and proposed his theory called 'Plasmogenia' in which he tried to recreate *in vitro* the chemical nature of life. During his work, he stressed the formation of chemical structures similar to cellular forms named 'colpoides' and 'sulfobios'. The abundance of sulfur in volcanic zones was an evidence for his sulfocyanic theory of the origins of life in which he suggested the use of ammonium thiocyanate and formaldehyde as raw materials for the synthesis of bio-organic compounds.

Although the amino acids are among the most abundant products formed in different prebiotic experiments, the formation of sulfur-containing amino acids presents problems due to their low yield of formation.

In order to highlight the importance of his work for the synthesis of amino acids using ammonium thiocyanate and formaldehyde, we reproduced some of his experiments performed on 1933. The results show that the total of amino acids was 2% of the initial thiocyanate used. Chromatograms using the HPLC technique reveal the presence of more than ten amino acids. Only glycine, alanine, cysteine, α -amino isobutyric acid and methionine were identified. Alanine was the most abundant amino acid in all samples of reactionated material analyzed.

CONTRIBUTED LECTURES

EXTRATERRESTRIAL ORGANIC MOLECULES

CONTRIBUTIONS OF ICY PLANETESIMALS TO THE EARTH'S EARLY ATMOSPHERE AND OCEANS

Akiva Bar-Nun¹ and Tobias Owen²

¹ *Department of Geophysics and Planetary Sciences, Tel Aviv University, Tel Aviv 69978, Israel;*

² *University of Hawaii, Institute for Astronomy, Honolulu, Hawaii 96822, USA.*

What can we learn from Earth Ocean's HDO/H₂O ratio and the ratio of the noble gases Ne/Ar/Kr/Xe on the cometary contribution to the oceans and atmosphere? Laboratory experiments on the trapping of gases by ice forming at low temperatures implicate comets as major carriers of the heavy noble gases to the inner planets. These icy planetesimals may also have brought the nitrogen compounds that ultimately produced atmospheric N₂. However, if the sample of three comets analyzed so far is typical, the Earth's oceans cannot have been produced by comets alone, they require an additional source of water with low D/H. The highly fractionated neon in the Earth's atmosphere may also indicate the importance of non-icy carriers of volatiles. The most important additional carrier is probably the rocky material comprising the bulk of the mass of these planets.

Yet, both the HDO/H₂O ratio and the noble gases ratios suggest a major cometary contribution to Earth's oceans and atmosphere. If comet P/Halley is typical, the ratio of water to organics is about 4:1. Thus a huge amount of organics came to Earth on comets. Whether they survived the entry and impact is another question.

FORMATION AND MODIFICATION OF AMINO ACID PRECURSORS IN SPACE ENVIRONMENTS

Kensei Kobayashi,¹ Yoshinori Takano,¹ Takeo Kaneko,¹ Hirofumi Hashimoto,² Takeshi Saito,³ Seiji Sugita,⁴ Kazumichi Nakagawa,⁵ Tairo Oshima⁶ and Masamichi Yamashita⁷

¹ *Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan (E-mail: kkensei@ynu.ac.jp);* ² *Institute of Engineering Mechanics, University of Tsukuba, Tsukuba 305-8573, Japan;* ³ *Institute for Advanced Studies, 1-29-6 Shinjuku, Shinjuku-ku, Tokyo 160-0022, Japan;* ⁴ *Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan;* ⁵ *Faculty of Human Development, Kobe University, Nada-ku, Kobe 657-8501, Japan;* ⁶ *Faculty of Life Sci., Tokyo Univ. of Pharm. and Life Sci., Hachioji 192-0392, Japan;* ⁷ *Institute of Space and Astronautical Science, Sagami-hara 229-8510, Japan*

A wide variety of organic compounds have been detected in extraterrestrial environments. Among them, those found in meteorites and comets have become of interest as sources of Earth's first biosphere. It was supposed that organics in small bodies were formed and evolved in interstellar space. We performed laboratory simulation of organic formation and modification in space environments.

An ice mixture of carbon monoxide, ammonia and water was made in a cryostat at 10K to simulate ice mantle of interstellar dusts (ISDs). The ice was irradiated with high-energy protons from a van de Graaff accelerator or with UV photons ($\lambda > 120$ nm) from a deuterium lamp. After irradiation, volatile products were analyzed

with a quadrupole mass spectrometer, and non-volatile products were analyzed by HPLC after acid-hydrolysis.

Amino acids such as glycine, alanine and β -alanine were detected in the hydrolysates of the products of UV-irradiation, as well as those of proton-irradiation [1]. Apparent G-value of glycine by UV was 2.7×10^{-5} , which was 3 orders of magnitude smaller than that from a gas mixture of the same components. This difference seems to be mainly contributed to the fact that carbon monoxide might easily escape from the ice mixture during irradiation: When a mixture of methanol, ammonia and water was used, the ice mixture and the gas mixture gave almost the same G-values of glycine.

It is suggested that both UV and cosmic rays can easily form amino acid precursors in ISDs. In space, wider range of UV light, including extreme UV light, are available. It will be of great interest to test the effects of such high-energy UV on the formation and modification of bioorganic compounds by using an exposed facility of the space station.

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PHOTOPRODUCTION OF CHIRAL AMINO ACIDS UNDER SIMULATED INTERSTELLAR CONDITIONS

Uwe J. Meierhenrich¹, Guillermo M. Munoz Caro², Willem A. Schutte², Bernard Barbier³, Almudena Arcones Segovia², Helmut Rosenbauer⁴, Wolfram H.-P. Thiemann¹, André Brack³

¹ *Universität Bremen, Physikalische Chemie, Leobener Str., D-28359 Bremen, Germany;* ² *Raymond and Beverly Sackler Laboratory for Astrophysics at Leiden Observatory, P.O. Box 9513, 2300 Leiden, The Netherlands;* ³ *Centre de Biophysique Moléculaire, Rue Charles Sadron, F-45160 Orléans, France;* ⁴ *Max-Planck-Institut für Aeronomie, Max-Planck-Str. 2, D-37189 Katlenburg-Lindau, Germany*

The interstellar medium ISM is known to be composed of both gas and dust particles. These dust particles are assumed to be made up of silicate grains, surrounded by an ice layer including carbon containing molecules. We simulated interstellar conditions in the laboratory in order to receive information on the interaction between the interstellar gas and interstellar dust particles. H₂O, CO₂, CO, CH₃OH, and NH₃ were deposited at 12 K and a pressure of 10^{-7} mbar onto a solid surface under irradiation of representative interstellar electromagnetic radiation. The ice layer developed on the solid surface was analysed by enantioselective gas chromatography and mass spectrometry GC-MS. In order to exclude contamination processes parallel experiments were performed with ¹³C-containing educts. After the analytical steps of extraction, hydrolysis, and derivatization 16 amino acids were identified in the simulated ice mantle of interstellar dust particles [1]. The

results were confirmed by the ^{13}C -experiments, definitely excluding contaminations. The chiral amino acids were identified as being totally racemic. The obtained results strongly suggest that amino acids had already been formed in the ISM. With the delivery of such precursor molecules through the bombardment of Earth with meteoritic resp. cometary material during the early history of Earth they might have contributed to prebiological reactions forming molecules being of crucial relevance for processes in chemical evolution.

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CHIRALITY AND MINERAL ASSOCIATION OF ISOVALINE IN THE MURCHISON METEORITE

Sandra Pizzarello¹ and Mike Zolensky²

¹ Arizona State University, Tempe, AZ85287 ²NASA Johnson Space Center, Houston TX77058, USA

Carbonaceous chondrites have been found to contain non-racemic amino acids and amines (1). The range and locales of formation of this chiral asymmetry have been subject to debate, however, studies conducted so far point to L-enantiomeric excesses (ee) that are limited only to compounds having an alkyl-substituted chiral carbon adjacent to the functional group(s).

Observed ee appear to vary between molecular species, various meteorites, and, for given a compound, even within the same meteorite; this variability extends also to the abundance ratios of asymmetry-carrying to racemic species. For example, meteoritic isovaline has shown ee that vary from 0–15% and ratios to alanine varying from 0.3–7.0.

Ee in meteorites have been attributed to possible photolysis by UV CPL. However, this process is strictly dependent upon the difference between coefficients of absorption for enantiomers in chiral compound, which limits the range of obtainable asymmetry. In view of the low anisotropy factor for amino acids, the CPL model appears inadequate to explain the range of ee observed in meteorites.

A comparative study was undertaken assessing the possible correlation between amino acid molecular and chiral distribution and petrological content in meteorites. Contiguous fragments of a Murchison stone were analyzed and organic analyses of the water extracts were coupled with XRD microscopy of the extracted powders, with focus on the main minerals serpentine and forsterite.

Results so far show a relation between calculated wt% of serpentine, i.e. matrix wt%, and relative abundance of isovaline, the most abundant homologue of the symmetry carrying α -substituted amino acids. The data appear to point to a secondary, possibly catalytic symmetry breaking process in locales of water alteration.

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PREBIOTIC SYNTHESIS

FORMATION OF AMIDE AND ESTER BONDS IN LIPIDS UNDER HYDROTHERMAL CONDITIONS

B. R. T. Simoneit and A. I. Rushdi

Environmental and Petroleum Geochemistry Group, College of Oceanic and Atmospheric Sciences, Oregon State University, Oregon 97331, U. S. A.

Lipids, consisting mainly of alkanolic acids and alkanols, with lesser amounts of alkanes, alkanones, alkyl esters and alkanals, can be synthesized by thermocatalytic reactions from formic or oxalic acids under aqueous high temperature and pressure conditions. This supports abiogenic lipid synthesis under hydrothermal conditions and raised the question about secondary product formation. Thus, we examined reductive dehydration (condensation) reactions in aqueous high temperature and pressure media. Experiments were performed to specifically assess the formation and alteration of various amide, ester and nitrile compounds from lipid precursors under hydrothermal conditions. Mixtures of palmitamide or palmitic (hexadecanoic) acid with water and oxalic acid, and with or without ammonium bicarbonate were heated at 300 °C for 24 and 72 hours. The results show that experiments with palmitamide in water yield palmityl palmitamide, palmitamide and palmitic acid as the dominant products. Mixtures of palmitamide, water and oxalic acid produced mainly methyl- and dimethyl alkanamides, methyl alkanooates and wax esters. Experiments with palmitamide, ammonium bicarbonate and water generated alkanamides (>50%), with significant amounts of methyl alkanamides, *n*-alkanenitriles and methyl alkanooates. Substituting an alkanedioic acid in the experiments yields alkanenitriles, methyl alkanamides, alkanedinitriles, amidoalkylamines and alkanediamides as the major compounds. Therefore, reductive condensation reactions occur under simulated hydrothermal conditions to form amide, nitrile and ester bonds. The chemistry and kinetics of these processes are under further study.

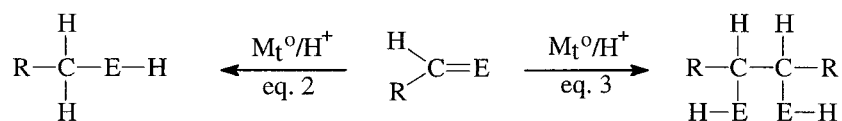
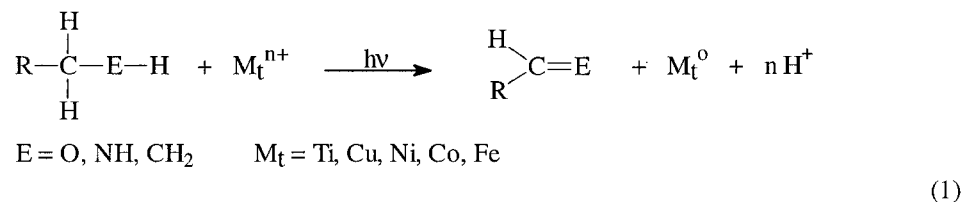
PHOTOCHEMICAL TRANSITION METAL-MEDIATED REACTIONS IN THE PREBIOTIC SYNTHESIS OF SUGARS

John J. Eisch, Peter R. Munson and John N. Gitua

Department of Chemistry, State University of New York at Binghamton, Binghamton, New York 13902-6016 U.S.A.

Ultraviolet light has long been recognized as the most abundant energy source on the Prebiotic Earth for organic synthesis. Photochemical processes involving redox reactions of transition metals such as Ti, Cu, Ni, Co and Fe possess the selective and versatile potential for having harnessed solar energy for useful prebiotic synthesis. Such irradiation can be captured by the photoreduction of the metal ions with the concomitant oxidation of simple alcohols, amines or even alkanes (eq. 1). The stored chemical energy from eq. 1 can be released in dark reactions (eqs. 2 and 3), whereby useful and novel C-H and C-C bonds would be formed. A cycle of these light and dark reactions, catalyzed by transition metals, could convert

simple organics, such as CH_3OH , CH_3NH_2 and CH_3CH_3 , into longer carbon chain derivatives, e.g. vicinal glycols, diamines, diimines and amino alcohols, which are appealing prebiotic precursors, especially for the generation of carbohydrates.



Precedents for the photochemical and thermal reactions of transition metals depicted in eqs. 1-3 are known (Eisch et al., 1994-2001). The capability and pertinence of these processes for the prebiotic synthesis of ribose and the emergence of an RNA-World will be delineated in the presentation.

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CARBON MONOXIDE CLUSTERS IN THE FORMATION OF D-SUGARS, L-AMINO-ACIDS, RIBONUCLEOTIDES AND DE-OXYRIBONUCLEIC ACIDS IN PREBIOTIC MOLECULAR EVOLUTION ON EARTH

Nigel Aylward and Neville Bofinger*

School of Natural Resource Sciences, Queensland University of Technology, George St. Brisbane, Queensland 4000, Australia. (Corresponding author)*

Carbon monoxide clusters oriented on a template of porphyrin are postulated to be the precursors of modern sugars. The simplest magnesium-porphyrin-carbon monoxide adduct is a van der Waals complex that can be approximately considered under the point group C_{4v} . The excitation of this adduct in a prebiotic type of photosynthesis by an allowed $\text{A} \rightarrow \text{E}$ electronic transition of 0.23 eV allows the carbonyl group to move to the periphery of the ring system with an activation energy of 0.19 eV, where it resides as a transition state.

This process can be repeated a second time, after which both transition states can react through the porphin conjugated bonding enabling the activation energy to be trapped as chemical energy. This process can be repeated until all transition sites are filled, and reaction has occurred to give rise to D-sugars, preferentially. The activated carbonyl group can react with other ligands held by the magnesium ion, such as imines to form aziridones that lead by hydrolysis to L-amino-acids. The activation energy to excite the peripherally held carbonyl group is 0.23 h which is sufficient to overcome the activation energy of 0.08 h.

The Lamor precession of the p electrons under the influence of the implied radiation field is considered to be the cause for the carbonyl group to select one resonance form of the magnesium-porphin preferentially.

The cluster has an initial keto group which allows nucleoside formation.

Reaction of the cluster with metaphosphite ion gives ribonucleotide. However, the cluster can also lose hydroxyl groups and become hydrogenated to form cyclic 3'-5' phospho-deoxyribonucleotide which should polymerise to nucleic acid. The further formation of hexoses and heptoses is considered.

The reactions have been shown to be feasible from the overall enthalpy changes in the ZKE approximation at the HF and MP2 /6-31G* level.

NON-ENZYMATIC TEMPLATE-DIRECTED LIGATION OF SHORT-CHAINED 2'-5' or 3'-5' OLIGORIBONUCLEOTIDES

Hiroaki Sawai, Makoto Wada, Tukasa Kouda, Hiroaki Ozaki

Department of Chemistry, Gunma University, Kiryu, Gunma, 376 Japan

It has been proposed that RNA played the roles of information carrier and catalyst at the prebiotic era. Contemporary RNA has exclusively a 3'-5' phosphodiester bond. However, 2'-5' linked oligoribonucleotides are chemically feasible and are formed in model processes of prebiotic synthesis of RNAs. Thus 2'-5' and 3'-5' linked oligoribonucleotides could have been formed during chemical evolution. In the later stage, the 3'-5' linked RNA could be selected due to its slower rate of hydrolysis, its higher helix forming ability, and/or its flexibility to form various conformation such as loop, stem and hairpin structure, compared to the corresponding 2'-5' linked RNA. The non-enzymatic replication efficiency is also an important factor for the selection. Previously, we have shown that 2'-5' diadenylate condenses each other forming oligoadenylates more efficiently on 2'-5' decauridylylate template than on 3'-5' linked decauridylylate template, although complexes are formed completely in both cases under the reaction condition. Here we report ligation of mixed sequence of 2'-5' or 3'-5' linked tetramers (5'-pACUG-3') on 2'-5' or 3'-5' linked complementary decaribonucleotides (5'-CAGUCAGUCA-3') template. The CD and UV melting studies showed that the helix formation took place in the possible four combinations between linkage isomers of the substrate tetramer and the template decamer ([2'-5' vs 2'-5'], [2'-5' vs 3'-5'], [3'-5' vs 2'-5'] and 3'-5' vs 3'-5']) under the ligation condition. The tetramers condensed each

other on the complementary decaribonucleotide template forming the corresponding octamer. However, the ligation efficiency varied largely depending on the type of linkage of the substrate and the template. The yield of the octamer by the template-directed ligation is in the following order: $[2'-5' \text{ vs } 2'-5'] > [3'-5' \text{ vs } 3'-5'] > [2'-5' \text{ vs } 3'-5'] > [3'-5' \text{ vs } 2'-5']$. The resulting linkage of the octamer formed from the $2'-5' \text{ vs } 2'-5'$ system is mainly $2'-5'$.

MONTMORILLONITE, OLIGONUCLEOTIDES, RNA AND ORIGIN OF LIFE

Gözen Ertem

Georgetown University, Lombardi Cancer Center, E420 The Research Building, 3970 Reservoir Road, N.W. Washington, D.C. 20007, USA

As was proposed by Bernal, minerals might have served as catalyst for the formation of bio-organic molecules on the primitive Earth (1949). Research carried out in J. P. Ferris' Laboratory to test this hypothesis demonstrated that montmorillonite, a clay mineral with layer structure, catalyses the self condensation of activated mononucleotides, ImpNs, in aqueous electrolyte solution (Ferris and Ertem, 1992; Kawamura and Ferris, 1994; Prabahar *et al.*, 1994; Ding *et al.*, 1996). Formation of phosphodiester bond takes place mainly between the basal surfaces of montmorillonite (Ertem and Ferris, 1998). While oligocytidylates formed by montmorillonite catalysis serve as template for the oligomerisation of 5'-ImpG (Ertem and Ferris, 1996), oligouridylates do not catalyse formation of oligoadenylates (Ding *et al.*, 1996).

Montmorillonite catalysed reaction of binary and quaternary mixtures of ImpNs produced both homo- and hetero-dimers with significant regio- and sequence selectivity (Ertem and Ferris, 2000). Reverse phase HPLC elution profiles of trimer fractions formed in the montmorillonite-ImpA-ImpN (N=C or U) reaction and hydrolysed with alkaline phosphatase, and the trimer fraction formed in base hydrolysis of Poly(A,C) or Poly(A,U) and hydrolysed with alkaline phosphatase were compared. The highest number of homo-trimers that can form in the clay catalysed reaction of binary monomer mixture is eight. Presence of more than eight fractions on the chromatogram strongly suggest the formation of hetero-trimers along with homo-trimers.

In the light of these findings, significance of mineral catalysis in the course of processes leading to the origin of life will be discussed.

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NON-ENZYMATIC RNA POLYMERIZATION IN EUTECTIC PHASES IN ICE

Pierre-Alain Monnard*, Anastassia Kanavarioti and David W. Deamer

Department of Chemistry and Biochemistry, University of California, 1156 High street, Santa Cruz, CA 95064, USA (To whom correspondence should be addressed. E-mail: monnard@chemistry.ucsc.edu).*

The RNA-world hypothesis requires an efficient non-enzymatic polymerization of RNA from monomer solutions that were likely to be very dilute(1). Two main hurdles have precluded efficient polymerization: the presumed low availability of organic building blocks on the Early Earth and the poor non-enzymatic incorporation of uridylic acid into polymers(2, 3).

Using eutectic phases in ice at $-18\text{ }^{\circ}\text{C}$ to concentrate dilute monomer solutions, we have found that the formation of homopolymers from phosphoimidazole-activated uridine is facilitated in the presence of low concentrations of Pb^{2+} and Mg^{2+} . Product yields corresponded to a monomer incorporation of 80–94% into oligouridylates up to 14-mers with 30% of the oligomers having one or more 3'-5' linkages. The increased concentration of solutes in the eutectic phases accelerated the reaction and diminished the hydrolysis/deactivation of the activated monomer.

When mixtures of the four nucleobases (ImpN) were used, random oligomer fragments were synthesized under these conditions. The nucleobase analysis of the products established that all four bases were incorporated. Moreover, the amount of each nucleobase in the product was a function of its relative concentration in the initial mixture, supporting the proposition that incorporation of each base proceeded at *comparable rates*.

Synthetic reactions could therefore take place in an ice matrix without requiring transient melts produced by large cometary or meteoritic impacts(5). Thus, Archaean ice deposits, at the poles or at higher altitudes, as on the contemporary Earth, could have provided a broad niche for synthesis of biopolymers or their precursors on the early Earth.

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THE TRANSITION FROM COMPOSITIONAL TO POLYMER-BASED REPLICATION

Barak Shenhav, Ran Kafri, Doron Lancet

Dept. Molecular Genetics, the Weizmann institute of Science, Rehovot, Israel

The basic Graded Autocatalysis Replication Domain (GARD) model consists of a repertoire of monomeric molecules (typically amphiphiles), which join and leave a non-covalent assembly. Its replication-like behavior is governed by mutual catalysis, determined by the assembly's composition (Lancet *et al.*, this volume). One of the drawbacks of the basic GARD model is the lack of a clear path from GARD's 'compositional genome' towards 'sequential genomes' with template-dependent replication. We have now explored an extension (polymer GARD or P-GARD), in which oligomers may form on a monomer-based GARD 'scaffold'.

In P-GARD, polymers form as a result of internal chemical rules, rather than being pre-assumed as in other prebiotic models. P-GARD includes differential equations that represent ligation in addition to join/leave reactions. Its catalytic parameters for covalent bond formation are computed by template-like complementation among oligomers. Testing this concept with monovalent monomers that can only form dimers, we have observed 'take-over' of dimers, and the formation of compositionally stable, replication-prone assemblies with both monomers and dimers. Partial autotrophy prevails, whereby only simple monomers are externally supplied, and all the more complex oligomers are internally generated, in a manner resembling biosynthetic metabolism.

We are currently exploring the behavior of P-GARD systems with longer oligomers. As P-GARD reactions are reversible, oligomer length distribution will be self-limiting, with no exceedingly long 'tar-like' chains. Yet, because there is no limit on oligomer complexity, the expected evolutionary scenarios are open-ended.

The P-GARD model requires vast computing resources, hence we intend to harness the massive home computing power provided by the OOL@home project, to simulate the long-term evolution of P-GARD assemblies into entities that might resemble protocells.

EVOLUTIONARY GENOMICS

MONOMER WORLD

Robert Shapiro

Department of Chemistry, New York University, New York, N.Y. 10003, USA (rs2@nyu.edu)

Although many theories propose that life began with a replicator, the spontaneous formation of such a substance through abiotic processes would be very improbable. Proposed replicators feature a uniform backbone, with a limited number of information-carrying substituents. The polymerization of complex abiotic mixtures, by contrast, would be expected to provide products with a nonrepetitive backbone, whose length would be limited by chain-termination processes, and linearity compromised by branching. In accord with these expectations, the macromolecular carbon fraction of meteorites presents an irregular, heterogeneous structure, featuring condensed ring systems with aliphatic cross-links. (Cronin and Chang, 1993)

We have analyzed the difficulties involved for the specific case of abiotic amino acid polymerization (Shapiro, 2000); we now extend this analysis to consider peptide nucleic acids (Nelson *et al.*, 2000) and proposed RNA predecessors based on a regular sugar-phosphate backbone (Herdewijn, 2001). No reason exists to expect the formation of such substances outside of a laboratory or living cell.

These arguments support the conclusion that life began with monomer interactions, and that biopolymers arose later in evolution. In this monomer world, reactions were facilitated by small biocatalysts (Kochavi *et al.*, 1997) and information was stored in a compositional genome (Segré and Lancet, 2000).

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AMINOACYL-TRNA SYNTHETASES AS MARKERS OF GENETIC CODE DEVELOPMENT

Luís Ribas de Pouplana and Paul Schimmel

Skaggs Institute for Chemical Biology and Departments of Molecular Biology and Chemistry, The Scripps Research Institute

Aminoacyl-tRNA synthetases (ARS) catalyze the specific aminoacylation of tRNA molecules with their cognate amino acids, a crucial step in the process that translates genetic information to protein sequence. Their high level of sequence con-

ervation suggests that the evolutionary events that generated these enzymes took place before the establishment of the three phylogenetic branches of life (Bacteria, Archaea, and Eukarya).

Despite the close functional relationship between the aminoacylation activities of tRNA synthetases and the genetic code, attempts to relate the evolution of these enzymes to the development of the code have been inconclusive. Recent realizations concerning the structural relationships between different classes of ARS have offered a new insight into the role played by these enzymes in the development of the genetic code machinery [1].

In particular, the synthetases may have developed in pairs that evolved as tRNA binding proteins, and followed the increase in complexity of the tRNA molecules. Thus, the extant families of synthetases are a product of the development of the genetic code, and their structural relationships may reflect the nature of intermediate steps in the establishment of the codon-amino acid relationships [2].

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NOVEL INTRONS FROM SUBSURFACE BACTERIA: STRUCTURAL FEATURES AND PHYLOGENIES

Alexey Veprikskiy, Inna Vitol and Sandra Nierzwicki-Bauer
Department of Biology, Rensselaer Polytechnic Institute, Troy, NY 12180

Two subsurface bacterial isolates, belonging to the γ - and β -proteobacteria genera of *Pseudomonas* and *Comamonas*, have been identified to possess group I introns in their tRNA^{Leu}(UAA) and tRNA^{Ile}(CAU) genes respectively. Sequences of both introns have group I conserved elements, and they fold into a bona fide group I secondary structure. Both introns contain the 13-nt motif [GCCAAG C ... GAA GGU] in the P6 region, which is found universally conserved in bacterial introns. The tRNA^{Leu}(UAA) intron possesses the 11-nt motif [CCUACG ... UAUGG] in its P8 region, a structural feature not being common in bacterial introns but abundant in introns from eukaryotes. Structural similarity between the two motifs might suggest that they are similar functionally and both were originally present in bacterial introns. The tRNA^{Ile}(CAU) intron shares sequence and structural features characteristic to two group I subgroups, IC3 and IA1, the former being represented by bacterial and plastid introns, while the latter – by mitochondrial and plastid introns. It also possesses the 16-nt motif [ACCCUAACG ... CGAGGGU] in its P8 region, a universally conserved feature in cyanobacterial tRNA^{Leu}(UAA) and

tRNA^{fMet} introns. The cyanobacterial tRNA^{Leu}(UAA) intron is believed to originate in the common ancestor of the group and be the most ancient intron yet found. Plausibly, the 16-nt motif could date back to this time as well, and might be originally present in all bacterial introns. Phylogenetic analysis suggests that both introns have originated by horizontal transfer. The *Pseudomonas* intron is unrelated to cyanobacterial tRNA^{Leu}(UAA) introns but shares a common ancestor with the tRNA^{Arg}(CCU) introns from α -proteobacteria. The *Comamonas* intron clusters with the tRNA^{Ile}(CAU) intron from the β -proteobacterium *Azoarcus*, showing, at the same time, a close affinity to plastid IA1 introns interrupting photosynthesis genes.

EXPLORATION OF SUBSURFACE PACIFIC MICROBIAL ECOSYSTEMS

Christopher H. House¹, Nils Holm², and the ODP Leg 201 Science Party

¹ Penn State Astrobiology Research Center & Department of Geosciences, Pennsylvania State University, 208 Deike Building, University Park, PA 16802 USA chouse@geosc.psu.edu; ² Department of Geology and Geochemistry, Stockholm University, SE-106 91 Stockholm, Sweden nils.holm@geo.su.se

The Ocean Drilling Program Leg 201 was carried out during February-March 2002. It addressed several fundamental questions about the deeply buried biosphere. Both deep-sea and shelf sediments were cored and recovered at a series of sites in the eastern equatorial Pacific, the Peru Basin, and the Peru Margin with the purpose of exploring the distribution, activities, community structure, phylogenetic affinities, and global biogeochemical consequences of microbial communities buried beneath the seafloor. Subsurface sedimentary environments explored during Leg 201 included (1) sulfate reducing, sulfate depleted methanogenic, and sulfate-reducing methanotrophic zones of the Peru coastal margin; (2) open-ocean sulfate-rich sediments with sulfate reduction, methanogenesis, and methanotrophy; and (3) open-ocean sulfate-rich sediments where manganese reduction occurs (Peru Basin). The coastal-margin sites included both shallow-water sites where most of the methane is present as free gas and a subduction trench site where methane hydrates occur. The cruise revealed a microbial subsurface ecosystem often dependent on the past oceanographic conditions, as well as geochemical interfaces resulting in subsurface microbial rich layers. Post-cruise research will be directed in part on studying this microbial biosphere through both culture-dependent and culture-independent methods.

SER-HIS: THE MINIEST NUCLEASE AND PROTASE EVOLUTED FROM N-PHOSPHOSERINE AND HISTIDINE

Yuan Ma, Yufen Zhao

Department of Chemistry, School of life Science and engineer, The Key Laboratory of Bioorganic Phosphorus Chemistry of Ministry of Education, Tsinghua University, Beijing, 100084, China

Ser-His is the smallest peptide reported in the literature, which has the nucleic acids and proteins cleavage function [1]. In the early studies on interaction of N-phosphoamino acids and DNA, we found that after co-incubation at 37 °C for several hours, most N-phosphoamino acid could cleave DNA, but the further tests established it was because of the hydrolysis of N-phosphoamino acids. N-Phosphoamino acid hydrolyzed to form dialkylphosphoric acid, alkylphosphoric acid and phosphoric acid, which decreased the pH of the solution, and leded DNA to be cleaved.

When N-phosphoamino acids were dissolved in neutral buffer, they did not cleave DNA; the only exception was N-phosphoserine in saturated histidine buffer [2]. It was very strange that the fresh solution had no function to DNA, while after the solution was frozen storage in -20 °C for 20 days it showed such ability. Using FAB-MS (fast atom bombardment mass spectrometry) detecting, we found there were Ser-His and Ser-Ser dipeptides formed in the aged solution. Was it possible that the yielded dipeptides decreased DNA? After we used commercial Ser-His dipeptide to do experiments, it was confirmed DNA was scissored by Ser-His. The following research by our group and cooperators discovered Ser-His can cleave proteins, too.

In a brief summary, N-phosphoserine can formed Ser-His dipeptide with histidine even in -20 °C frozen condition, and the product has an important biochemical function. This probably is one of the paths in the early chemical evolution of life.

THE FOSSIL RECORD

FE ISOTOPE FRACTIONATION AND THE ORIGIN OF THE BANDED IRON-FORMATIONS

Paul S. Braterman*, Brian D. Brister, Brian Beard and Clark M. Johnson

*Department of Chemistry, University of North Texas, Denton, TX 76203, USA; Department of Geology and Geophysics, University of Wisconsin-Madison, Madison, WI 53706, USA (**author for correspondence; email: psb@unt.edu)*

Banded iron-formations are found in the earliest sediments, with particularly massive deposits dating from the period between 2.7 and 1.8 Bybp. The iron is commonly present as hematite or magnetite, implying oxidation of dissolved Fe(II) to Fe(III). The proterozoic banded iron-formations in particular have been found to show large shifts in the relative ratios of ^{54}Fe , ^{56}Fe and ^{57}Fe .

The oxidation of Fe(II) could have occurred by any of several different mechanisms, including

1. photooxidation
2. reaction with atmospheric oxygen
 - (a) thermal
 - (b) with photochemical promotion
3. direct biological oxidation

as well as combinations of these, such as reaction with biogenic oxygen.

We propose that Fe isotope shifts may offer a useful method for distinguishing between these possibilities, and that the variation of the shifts across the more extensive and best preserved deposits would also provide a test of the hypothesis that upwelling of Fe was the limiting factor in their formation.

ON THE DIVERSITY AND DISTRIBUTION OF EARLY ARCHAEOAN LIFE

Frances Westall

Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, 45071 Orléans cedex 2, France (westall@cnsr-orleans.fr)

The Barberton and Pilbara greenstone belts in South Africa and Australia, respectively, are the only two existing, Early Archaean, supracrustal terrains where the oldest morphological evidence for life can be studied. Sedimentological analysis of the environmental conditions represented by the >3.4 b.y.-old rocks of Barberton and the Pilbara document potential, habitable niches in shallow water, littoral, sub-aerial, hot spring, evaporitic and possible sodic lake environments. Hydrothermal activity was pervasive and affected all environments.

On the basis of macroscopic to microscopic study, I have documented the existence of microbial mats and microbial fossils in volcanoclastic sediments in shallow water, littoral, hot spring, evaporitic and possibly subaerial deposits. The microbial mats range from delicate veils ($\sim 1 \mu\text{m}$ thick) deposited in quiet waters to thick, robust films up to $10 \mu\text{m}$ thick that characterise the more exposed, intertidal to possibly subaerial environments. The mats form domal and, more commonly tabu-

lar, stromatolites. They include fossilised bacteria exhibiting filamentous structures of two types (<0.5 μm diameter, up to 10 μm long and 0.5–2.5 μm diameter, up to 200 μm long), rod-shaped morphologies (1 μm diameter, <3.8 μm long), and coccoidal forms (0.5–1.0 μm diameter). The hydrothermally-silicified bacteria were identified on the basis of morphological, colonial and chemical criteria. They probably represent thermophilic chemolithotrophs, heterotrophs and anoxygenic photosynthesisers. No morphological evidence was found for cyanobacterial fossils (the $-26\text{‰}\delta^{13}\text{C}$ value is also produced by anoxygenic thermophiles).

By 3.47 b.y. ago bacteria had already colonised many of the niches available (this study is still on-going). Moreover, there is very strong similarity between the forms found in both Barberton and the Pilbara. These two areas were not yet co-joined in this period. These facts argue for relative diversity, wide distribution, as well as a certain spatial homogeneity, probably aided by ocean current distribution since most of the Earth's surface was water covered.

HISTORY, PHILOSOPHY AND EDUCATION IN THE ORIGIN OF LIFE

ORIGIN OF LIFE: STUDIES IN HYPOTHESIS CONSTRUCTION

Zann Gill

*NASA Ames Research Center PO Box 242, Moffett Field, CA 94035-1000, 650-604-4370
sgill@mail.arc.nasa.gov; New Affiliation effective March 15, 2002, MetaVu Network,
650-917-1612, zanngill@metavu.net*

I focus on the strategies that a range of brilliant scientists use to generate hypotheses on the question, 'How did life originate on Earth?' In the absence of substantial evidence their 'logical' reasoning leads to wonderfully conflicting results. C.S. Peirce thought the terms 'deduction' and 'induction' did not fully cover the mental activity of the creative scientist, who must make an initial inferential leap to conceive a new hypothesis. Peirce coined a new term, 'abduction', to address how a new hypothesis is generated.

Definitions of life used by origin of life theorists fall generally into two overlapping sets. The first set of definitions looks at what living things are made of and how they function, at what life is from a series of biological perspectives: physiological, metabolic, biochemical, genetic. But others define life independent of its material components, by considering its underlying principles, limits and boundaries, and prerequisite contexts for life, including non-biological perspectives, from physics and the second law of thermodynamics to information theory and rules of behavior for closed versus open systems.

For the many definitions of life there are counterexamples that contradict the definition. If life and its origins could be explained in terms of life-as-we-know-it, we could stop with biology. But where information theory and abstract, non-material principles apply to the origin of life question, we are forced to juxtapose origin of life theorists with artificial life experimentalists who try themselves to originate life. I'll use the buttons-and-thread analogy, adopted by Stuart Kauffman for the origin of life, to characterize the associative connections of this abduction experiment. I will explore metalevel principles such as tolerance (the precise use of imprecision), autonomy, and the role of paradox in generating hypotheses about the origin of life.

THE ROLE OF LIFE IN THE COSMOLOGICAL REPLICATION CYCLE

B. A. Balazs

Department of Astronomy, L. Eötvös University, Budapest, Hungary

'The general theory of relativity established that it is the structure of four-dimensional spacetime which manifests itself as gravitation. With similar logic, a spacetime theory of all interactions would establish decisively that there exists nothing in the world but structured spacetime. Certain structures would manifest themselves as galaxies, others as quarks or black holes, flowers or bacteria, yet others would

be you or me. If this is really so, then it would surely have been an event of cosmic significance when spacetime became conscious of itself.”*

Life has existed on Earth for a full third of the lifetime of our universe. It is the most expressive and complete manifestation known of the universes cosmological capacity for complexity. Even so living beings are not traditionally conceived of as cosmologically relevant structures. Indeed:

- Life is considered as a frail insignificance as compared with the mighty forces of the gigantic cosmic objects.
- The whole biological evolution of the genetically coded living structures is looked upon as a stochastic process of random mutation and selective advantage, many of whose outputs are incidental and idiosyncratic.

In the present paper we put these conventional views in issue and venture upon to show that

- *life is not a blind chance* but an integral part of the universe, strongly favored by the physical laws and constants of inanimate nature;
- the *emergence* of increasingly intelligent life is a robust phenomenon, *plays an important role in the cosmological replication cycle*, and its manifestations should make up an important part of our world picture.

The advent of a *new (bio)cosmological era* will strongly stimulate and influence our thoughts on ourselves and on the world then will presumably result in a radical change of our view on the role of life in the cosmos. One has to adopt a strictly *super-Copernican*** standpoint: We got very far away from any anthropocentric vision of the universe, yet at the dawn of the third millennium we begin to realize, that life – as the most important manifestation of the natural *phenomenon of emergence* – is an essential component of the cosmos. *Life seems to be capable of attaining the capacity to engage in cosmological engineering* and to improve the ability of the universe to reproduce. As the chances are that our universe (a large, casually fairly but not entirely separated spacetime region) is only a minor part of the totality of physical entity (*multiverse*), *life and intelligence* (conscious spacetime?) *may play an important role in the cosmological replication cycle, and they may be predestined to pervade and dominate a whole ‘family’ of universes.*

(This paper was supported in part by the OTKA grant T034998.)

The full text of this contribution with all notes and references is available at the home page of the author: <http://astro.elte.hu/munkatars/munkah.html>

* Quotation from G. Szamosi (*The Twin Dimensions*, McGraw-Hill, New York, N.Y., 1986)

** J.A. Wheeler’s phrasing in his famous book *At Home in the Universe*, AIP Press, New York, N.Y., 1994

DEFINING “LIFE”Carol E. Cleland¹ and Christopher F. Chyba²

¹ *Department of Philosophy and the Center for Astrobiology, University of Colorado, Boulder, CO 80309 USA; Cleland@colorado.spot;* ² *Center for the Study of Life in the Universe, SETI Institute, 2035 Landings Drive, Mountain View, CA 94043 and Department of Geological and Environmental Sciences, Stanford University, Stanford, CA 94404 USA*

The question ‘What is life?’ is central to biology. But how should one go about answering it? A popular strategy is to try to define ‘life’. Unfortunately, every proposed definition faces problems, often in the form of robust counter-examples. We argue that this is not an accident. The idea that ‘life’ can be defined is misguided, resting upon confusions about the nature of definition and its capacity to answer fundamental questions about natural categories. Definitions specify meanings of terms by dissecting concepts that we already possess. This works fairly well for terms such as ‘bachelor’, ‘fortnight,’ and ‘chair’, which designate categories whose existence depends solely upon human interests and concerns. But it doesn’t work for terms such as ‘water’, ‘heat’ and ‘bird’, which designate *natural* categories—categories that are delimited by nature as opposed to human interests and concerns. We argue that a satisfactory answer to the question ‘what is life?’ requires a general theory of the nature of living systems. In the absence of such a theory, defining ‘life’ poses a problem analogous to sixteenth century scientists trying to define ‘water’ before the existence of molecular theory. The best they could have done is to define ‘water’ in terms of the sensible features (e.g., being cooling, tasteless, odorless, thirst quenching) used to recognize samples of water. But none of these features would have revealed that water is H₂O.

PROPOSAL FOR A UNIVERSAL DEFINITION OF LIFEPedro Ruiz-Mirazo¹, Juli G. Peretó² and Álvaro Moreno¹

¹ *Departamento de Lógica y Filosofía de la Ciencia, Universidad del País Vasco UPV/EHU, San Sebastián/Donostia, and* ² *Departament de Bioquímica i Biologia Molecular, Facultat de Ciències Biològiques, Universitat de València*

Life is a complex phenomenon that requires both individual self-producing/self-sustaining systems and a historical-collective organization of those individual systems. On these lines, we propose to define living beings as *autonomous* systems with *open-ended* evolution capacities, whereby: (i) *autonomy* involves a far-from-equilibrium system that constitutes and maintains itself setting up an organizational identity of its own, a functionally integrated unit, founded on the realization of endo-exergonic couplings between internal self-constructing processes as well as with other interactive processes connecting it to the environment, and (ii) *open-ended evolution* means a process along which some systems reproduce their basic constitutive-functional dynamics generating an unlimited variety of similar systems, of different forms of expressing that basic dynamics, in a way that the resulting organization is not subject to any pre-determined upper bound of complexity

(even if it certainly is to the restrictions established by a finite environment and by the universal physical laws). Furthermore, in structural terms, we suggest that all living systems must have a semi-permeable active boundary (i.e., a *membrane*), an energy transduction apparatus (a set of *energy currencies*) and, at least, two types of interdependent macromolecular components: some carrying out and coordinating directly self-construction processes (*catalysts*) and some others storing and transmitting information which is relevant to carry out efficiently those processes in the course of subsequent generations (*records*). It is only when these records become informational that the whole network of autonomous systems allows for an open-ended increase in the complexity of the individual agents. Thus, the basic-individual organization of biological systems necessarily comes to depend on being instructed by patterns whose generation and reliable transmission cannot be explained but take into account the global, historical network of autonomous agents. We conclude that a proper definition of life should consider both levels, individual and collective: living systems cannot be fully constituted without being part of the evolutionary process of a whole ecosystem.

ASTROBIOLOGY/EXO BIOLOGY

SEARCH FOR WATER IN COMETS AND EXOPLANETS. POSSIBLE DETECTION AROUND eps Eridani AND ups Andromedae

Cristiano Cosmovici,

Istituto Scienze Cosmiche e Planetarie, CNR, Roma, Italy; Massimo Teodorani, Stelio Montebugnoli e Giuseppe Maccaferri, Istituto di Radioastronomia, CNR, Medicina (Bologna), Italy

The impact of 21 fragments of Comet Shoemaker-Levy/9 with the Jovian Atmosphere in July 1994 gave astronomers the unique opportunity to study the Chemistry and Physics of a planet during a catastrophic impact and permitted the detection of the 22 GHz Water MASER emission line for the first time in the Solar System (1).

Our measurements have shown that this spectral line can be used as a powerful diagnostic tool for planetary search outside the Solar System, as comets are able to deliver huge amounts of water (about 50 billion tons per comet) in planetary atmospheres rising the probability of LIFE development.

Thus in 1998 we started a search program using a 132.000 multichannel spectrometer coupled with the 32 m dish of the Medicina radiotelescope.

Up to now about 70 giant exoplanets have been discovered by different groups using indirect methods, but no information about the atmospheres and chemistry of these planets was possible up to now.

In this contribution we will illustrate the feasibility and the technique used and the first targets for the search within 50 light years.

Moreover we are able to show now the possible detection of water in planetary systems around epsilon Eridani and upsilon Andromedae.

If confirmed, this would be the first discovery of water in a planetary atmosphere outside the Solar System.

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CAN TERRESTRIAL ORGANISMS SURVIVE THE 'EXTREME' ENVIRONMENTAL CONDITIONS ON MARS? THE SPACE EXPERIMENT MARSTOX

P. Rettberg¹, E. Rabbow², C. Panitz¹, G. Horneck¹, R. Wäsch³, H. Wänke⁴

¹ DLR, Institute of Aerospace Medicine, Koeln, Germany; ² RWTH Aachen, Institut für Flugmedizin, Aachen, Germany; ³ DLR, Institute of Space Sensor Technology and Planetary Exploration Berlin, Germany; ⁴ Max Planck Institute for Chemistry, Mainz, Germany

The early histories of Mars and Earth show some similarities during the period when life emerged on Earth. The experiment MARSTOX of the ESA FOTON M-1 mission, scheduled for the end of 2002, will be a preparatory step for the future exploration of Mars as another planet which has had the potential for the evolution

of life. The interference of Martian soil components and the intense and nearly unfiltered Martian solar UV radiation with terrestrial resistant microbial forms, bacterial spores of *Bacillus subtilis*, will be tested. Different types of Mars soil analogs will be used to determine on one hand their potential toxicity alone or in combination with solar UV (phototoxicity) and on the other hand their UV protection capability. Two sets of samples will be used under different cut-off filters to simulate the UV radiation climate of Mars and Earth. After exposure in space in the ESA hardware BIOPAN the survival of and mutation induction in the spores will be analyzed at DLR together with parallel samples from the corresponding ground control experiment performed at DLR. This experiment will provide new insights into the adaptation to environmental extremes on Earth or other planets which define the principal limits of life and at the same time bear the potential for the evolution and distribution of life.

**BIOLOGICAL POTENTIAL OF MARS: POSSIBLE RECENT SURFACE/
NEAR-SURFACE LIQUID WATER AT HIGH OBLIQUITY**

Bruce M. Jakosky, Ruth E. Ley, and Michael T. Mellon,

University of Colorado, Boulder, CO 80309-0392, USA

Mars appears to meet the environmental conditions necessary to support life deep below the surface (100s of m to several km) where temperatures are high enough to allow liquid water to exist. These regions are not readily accessible to *in situ* investigation, however, so we have searched for conditions that could allow liquid water to exist at or near the surface. During epochs of high obliquity, high-latitude and polar temperatures will increase. At 60° obliquity, the approximate highest plausible value (compared to the current value of 25°), temperatures of the polar ice or of ground ice in the top meter of regolith can be at or above -20 °C. Further, if a thin lag deposit of dust forms that can locally inhibit polar-cap sublimation, temperatures can be at or above 0 °C. Even at sub-freezing temperatures, a thin film of water can exist at the interface between ice and soil grains or between two ice grains. Above -20 °C, these thin films are adequate for mobilizing ions that can provide nutrients to organisms, and are large enough to physically hold organisms. Furthermore, metabolism in terrestrial organisms has been demonstrated at -10 °C and possibly to as cold as -20 °C. Thus, conditions at high obliquity appear to overlap with those that are capable of supporting life. Sufficiently high obliquities may have occurred within the last few 10⁷ years. Although there is no liquid water today that could support organisms, it is possible that the polar regolith or ice could hold dormant organisms, spores, or chemical or morphological remnants of organisms. Exploration of these regions could be done with a lander that could target a geographically distributed area, and with a drill that would need to go only 1-2 m below the surface.

TITAN ORGANIC CHEMISTRY: NEW DATA AND EXOBIOLOGICAL IMPLICATIONS

François Raulin¹, Yves Bénilan¹, Jean-Michel Bernard¹, Patrice Coll¹, Antoine Jolly¹, Marie-Claire Gazeau¹, Sandra Ramírez Jiménez^{1,2}, François Shindo¹, Véronique Vuitton¹, Rafael Navarro-Gonzalez² and Jean-Claude Guillemin³

¹ LISA, CNRS & Universités Paris 12 & 7, 94010 Créteil Cedex France; ² Instituto de Ciencias Nucleares, UNAM, Mexico D.F. 04510, Mexico; ³ Laboratoire de Synthèses et Activations de Biomolécules, CNRS, ENSCR, 35700 Rennes France

Since the discovery of a dense N₂-CH₄ atmosphere on Titan, the largest satellite of Saturn, several organic compounds have been detected – as expected – in this environment and many others are supposed to be present. Complementary approaches have been systematically followed to study the organic chemistry that is going on in this planet-size laboratory:

- experimental simulation in the laboratory, using methane-nitrogen mixtures models of Titan's atmosphere
- theoretical modeling, with the development, in particular, of 0-D to 3-D photochemical models
- direct observation of Titan's atmosphere by remote sensing techniques (before the availability of in situ analysis in the frame of the Cassini-Huygens mission).

Several new results have been obtained recently concerning different exobiological aspects of

Titan:

- discovery of water (in gas phase and at the ppb level) by remote sensing techniques
- observation of Titan's surface showing a non homogeneous environment
- studies of potentialities of the presence of living systems in spite of the low surface temperature.
- availability of tholins (laboratory analogues of atmospheric organic aerosols) which mimic accurately Titan's aerosols, and the determination of their spectroscopic and optical properties;
- development of new photochemical models, and determination of new spectroscopic and kinetic data of importance for modeling Titan's chemistry
- more recent discovery that the main O-containing organics in Titan's atmosphere should be oxirane (from new simulation experiments revisiting the O-chemistry of Titan)

These new data will be reviewed and the exobiological consequences will be discussed, in the frame of the arrival of the Cassini-Huygens mission in the Saturn system in 2004.

ROUND TABLES

ROUND TABLE I: PRIMITIVE TERRESTRIAL ENVIRONMENTS

MANTLE REDOX EVOLUTION AND THE RISE OF ATMOSPHERIC O₂

James F. Kasting¹, David H. Eggler¹ and Heinrich D. Holland²

¹ Department of Geosciences, Penn State University, University Park, PA 16802; ² Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138

Why did atmospheric O₂ rise around 2.3 Ga¹ when cyanobacteria appear to have arisen at least 400 m.y. earlier²? One possibility is that the volcanic sink for O₂ was initially larger and that the upper mantle became progressively oxidized as hydrogen escaped to space³. Kump et al.⁴ calculated the change in mantle oxygen fugacity, fO_2 , required to trigger the O₂ rise. They found that a decrease in fO_2 by 2 log units would be required to flip the system from oxidizing to reducing. However, data on the Cr and V content of ancient basalts^{5,6} rule out a change of this magnitude. Catling et al.⁷ proposed a related model in which the continents became more oxidized; however, the mechanism by which this happened was not specified.

A recent reanalysis of volcanic gas data by Holland⁸ could change this story. Volcanic gases appear to be significantly more H₂-rich than had been previously thought. A decrease by as little as 0.7 log units in fO_2 may be sufficient to flip the system from oxidizing to reducing. Such a change could easily have resulted from the oxidation of iron in spinel⁹ and pyroxene¹⁰. Thus, the original mantle redox theory may indeed be correct.

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PROCESSING OF ORGANIC MATERIAL DURING COMETARY IMPACTS: AN EXPERIMENTAL PERSPECTIVE

Jennifer G. Blank

blank4@llnl.gov, LLNL, L-415, Livermore CA 94551

Given the abundance of organic material present in comets and the bombardment history of the early earth, what role did these primordial ice-balls play in bringing the building blocks of life to the surface of our planet? Here we use hyper-velocity impact guns to achieve in the laboratory the pressures of a cometary collision with

a rocky surface of the earth. We dope aqueous solutions with mixtures of organic materials and seal approximately 150 μl inside a 25.4 mm diameter \times 15 mm tall stainless steel canister, which is then mounted on its side in a $\sim 6 \text{ m}^3$ steel experiment tank. Disk-shaped, metal projectiles, accelerated to velocities of 0.5–1.8 km/s, impact the canister head-on and send a shock wave pulse through the container, generating pressures of up to nearly 40 GPa. These peak pressures have durations of several microseconds. Recovery of the liquids following an experiment and subsequent analysis using LCMS allow us to evaluate their degree of chemical change. We (1) determine the degree to which our starting materials survive and (2) characterize the reaction products formed as a consequence of the impact.

In earlier investigations, we demonstrated the survival of significant fractions of amino acids in aqueous and icy mixtures in these types of laboratory impacts; here we quantify the degree to which these initial building blocks polymerize to form peptides. We compare the effect of five particular side chains on the relative reactivities of the initial amino acid. We compare our experimental findings with results predicted using molecular dynamic simulations of amino acid polymerization in aqueous solutions at one atmosphere. Our results will be presented in the context of the prebiotic inventory of organic materials on the earth.

TRACING LIFE IN THE EARLY ARCHEAN: THE 3.8 Ga ISUA SUPRA-CRUSTAL BELT, SOUTHERN WEST GREENLAND

Mark van Zuilen¹, Aivo Lepland², Gustaf Arrhenius¹

(1) *Scripps Institution of Oceanography, University of California San Diego 9500 Gilman Drive, La Jolla CA 92093 – 0236*; (2) *Geological Survey of Norway, Leiv Eirikssonsvei 39, 7491 Trondheim, Norway*

During the first couple of hundred million years in Earth history, episodes of intense bombardment may have led to multiple ocean condensation-evaporation cycles. The last period of intense bombardment, that could have frustrated the presence of early life on Earth, culminated at approximately 3.9 Ga ago as evidenced by the lunar cratering record. The first evidence of water-lain sediments on Earth is found in the 3.8 Ga Isua Supracrustal Belt (ISB) in southern West Greenland. The ISB contains banded iron formations (BIFs) and pillow lava basalt, indicating a marine depositional setting. The fine banding in the BIFs further suggests a low-energy environment, very different from the presumed earlier impact-dominated environment. The rocks in the ISB have experienced a high grade of metamorphism ($P = 5 \text{ kBar}$, $T = 500\text{C}$) preventing preservation of morphological fossils. Interpretation of traces of life in the ISB has been drawn from carbon isotopic signature of graphite, believed to represent the alteration product of organic matter. Relatively graphitic carbonate rich Isua rocks that at the time were interpreted as shallow marine sediments gathered most attention in original early life studies. However, it has recently been shown that most, if not all carbonate rocks in the ISB have formed deep in the crust by interaction of hot secondary fluids with older supracrustal rocks

(metasomatism), and are therefore irrelevant for tracking traces of 3.8 Ga life. We present petrographic, mineralogic, and isotopic evidence that shows that the graphite in these metacarbonate rocks formed from the thermal disproportionation of iron carbonate, according to the reaction $6 \text{FeCO}_3 = 2 \text{Fe}_3\text{O}_4 + 5\text{CO}_2 + \text{C}$. These observations call for a reassessment of previously presented evidence for ancient traces of life in rocks of the Isua Supracrustal Belt.

ROUND TABLE II: PREBIOTIC SYNTHESIS: PAST, PRESENT, FUTURE

RADICALS, IONS AND MOLECULES IN CHEMICAL EVOLUTION

Mohindra S. Chadha

C6, Beach House, Juhu, Mumbai (Bombay) 400 049, India (E-mail: Chadhams@mail.com)

An area of great interest in chemical evolution research is the comparison of results of laboratory experiments with the more recent finding related to the chemistry of interstellar medium (ISM), comets, meteorites, planets and their satellites.

The occurrence and formation of hydrogen and nitrogen, methane and ammonia, hydrogen cyanide, carbon monoxide, formaldehyde, cyanoacetylene, aminonitriles etc and reactions of some of these molecules as such or in combination was presented at the 4th Trieste Conference on Chemical Evolution (Chadha, 1996).

An attempt will be made to understand some of the mechanisms which may be operating in the formation of organic components in the ISM and relate these to the chemistry of comets, meteorites, other planets, satellites and the laboratory simulation experiments.

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CHALLENGES IN 'PREBIOTIC' CHEMISTRY

Ramanarayanan Krishnamurthy

The Skaggs Institute of Chemical Biology at The Scripps Research Institute, 10550 North Torrey Pines Road, MB16, La Jolla, CA-92037, USA

Understanding the criteria, by which Nature has chosen nucleic acids as its genetic system, in chemical terms, is the goal of chemical etiology of nucleic acid structure and at the heart of our research in Scripps laboratory. Supplementing and complementing the understanding gained through the above approach, is the investigation into the questions pertaining to the origins of the individual components of nucleic acids, i.e., the sugars, the phosphate backbone and the nucleobases, which belongs to the realm of what is defined broadly as 'prebiotic' chemistry.

The talk will attempt to illustrate the approaches of some of these investigations, focusing on the long-standing problem of phosphorylation, which is a much-debated conundrum. The constraints, in coming to a conclusion regarding the 'prebiotic' nature of the molecules and the processes involved, will be discussed.

RADIOLYTIC DECOMPOSITION OF AMINO ACIDS IN CARBONACEOUS CHONDRITES

Jeffrey L. Bada and Gerhard Kminek,

Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0212 (jbada@ucsd.edu)

Analysis of carbonaceous chondrites has shown that these meteorites contain an abundant and diverse suite of amino acids and other organic compounds. This has led to the suggestion that organic compounds required for the origin of life could have been provided by the infall of organic rich material to the Earth. Amino acids are of particular interest because they are the building blocks of proteins and are thus essential to life as we know it. Amino acids are well preserved in the cold and dry environments characteristic of the asteroid belt and would not have undergone any appreciable thermal decomposition such as their formation. However, amino acids and other organic compounds have been exposed to ionizing radiation from the decay of radionuclides in the parent body and to the Solar Cosmic Radiation (SCR) and Galactic Cosmic Radiation (GCR) during their residence time on the parent body surface and during transit from the asteroid belt to Earth. We have recently determined the radiolysis decomposition constants of several dry amino acids. We estimate that a significant amount of radiolytic decomposition of amino acids could have taken place since their original formation nearly 4.5 billion years ago. The extent of radiolysis caused decomposition of amino acids increases with their Van der Waals, so the extent of decomposition increases with the molecular weight of the compound. This suggests that Murchison type material infalling the early Earth could have thus delivered much more amino acids, especially higher molecular weight amino acids, than is estimated from the present day amino acid abundances. The radiolytic decomposition of other types of organic compounds in Murchison type meteorites will also be discussed.

PREBIOTIC SYNTHESIS: PAST, PRESENT, FUTURE

Alan W. Schwartz

Evolutionary Biology Research Group, University of Nijmegen, The Netherlands

Much has been accomplished in the field of prebiotic synthesis since Miller's innovative experiment. It is often claimed that most of the 'key ingredients' (i.e. monomers) of living systems have been synthesized in simulated prebiotic experiments. This statement, however, does not provide an accurate picture of the current status of the problem. Even if we assume that the composition of the primitive atmosphere was favorable for synthesis, and overlooking the fact that entirely convincing syntheses have not yet been described for all compounds sought, serious problems remain. The most vexing of these is the often inescapable production of closely related and chemically similar sets of isomers and homologues; yielding a self-inhibiting set of reactants for stages of assembly to follow. Fortunately, a small

but growing class of selective reactions is becoming known, which holds out hope that these problems will eventually be solved. But what is next?

ROUND TABLE III: THE FIRST CELLS

SELF-ASSEMBLY OF ORGANIC MOLECULES AND THE ORIGIN OF CELLULAR LIFE

David W. Deamer

*Department of Chemistry and Biochemistry, University of California, Santa Cruz CA 95064,
deamer@hydrogen.ucsc.edu*

All membranous boundary structures in contemporary forms of life incorporate a lipid bilayer as the primary barrier to free diffusion of solutes. Bilayer-forming molecules are amphiphilic, with a hydrophilic 'head' and a hydrophobic 'tail' on the same molecule. Were amphiphilic molecules present in the mixture of organic compounds available on the early Earth? In past work, samples of the Murchison meteorite were extracted in an organic solvent (Deamer and Pashley, 1989). When this material interacted with aqueous phases, one class of compounds with acidic properties was clearly capable of forming membrane-bounded vesicles. In more recent work (Dworkin *et al.*, 2001) similar organic compounds were synthesized in precometary ices simulating those present in dense molecular clouds. These observations make it plausible that membrane-bounded structures were present at the time of life's origin.

We are now developing laboratory models of simple cellular systems with encapsulated macromolecules. Apel *et al.* (2002) recently demonstrated that mixtures of fatty acids and fatty alcohols having relatively short chain lengths (8–10 carbons) can form stable vesicles and encapsulate a functional enzyme. More complex systems incorporating both a catalytic polymerase and a DNA template have been prepared, and RNA synthesis in the vesicles has been observed (Monnard and Deamer, 2002). These results suggest that amphiphilic hydrocarbon derivatives were present on the early Earth and could self-assemble into boundary structures that were sufficiently permeable to allow passage of ionic substrates required for polymerization of macromolecules such as RNA, yet maintain those macromolecules within the boundary. Compartmentalization allows evolutionary selection of complex molecular groupings to occur, a process that could not as easily take place in mixtures of molecules free in solution.

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ON THE ORIGIN AND EVOLUTION OF METABOLIC PATHWAYS

Renato Fani

Dipartimento di Biologia Animale e Genetica, Via Romana 17-19, 50125 Firenze, Italy

The building up of metabolic pathways represented a crucial step in molecular and cellular evolution. In fact, if the Oparin's idea on the origin of life is correct, we can imagine that the exhaustion of the prebiotic supply of amino acids, bases, and other compounds must have imposed an important pressure favouring those primordial heterotrophic cells which became capable of synthesizing those molecules. Thus, the emergence of biosynthetic pathways allowed primitive organisms to become increasingly less-dependent on exogenous sources of organic compounds.

Several different theories have been suggested accounting for the establishment of metabolic routes. These explanations include: i) the retrograde hypothesis (Horowitz, 1945; 1965) according to which the present biosynthetic pathways were organized stepwise and backwards from the final metabolites of the pathways; ii) the possibility that at least some biosynthetic routes evolved forwards (Granick, 1965); iii) the idea that metabolic pathways appeared as a result of the gradual accumulation of mutant enzymes with minimal structural changes (Waley, 1969); and iv) the patchwork theory, according to which metabolic routes are the result of the serial recruitment of relatively small, inefficient enzymes endowed with broad-specificity that could react with a wide range of chemically related substrates (Ycas, 1974; Jensen, 1976).

The origin and evolution of metabolic pathways can be studied by sequence comparisons and by the 'directed evolution' experiments. The comparative analysis of different metabolic routes (nitrogen fixation, bacteriochlorophyll and histidine biosynthesis) suggested that: i) in the course of molecular evolution different mechanisms might have concurred in the arising of new metabolic abilities; ii) gene duplication is a major force in genome evolution and paralogous duplications of DNA stretches may have played an essential role in shaping the main metabolic pathways during the early stages of molecular evolution; iii) duplication may concern gene portions, coding for protein domains and motifs, entire genes, and entire operons.

THIAMIN BIOSYNTHETIC GENE EXPRESSION IN BACTERIA: AN ANCIENT MECHANISM FROM THE RNA WORLD?

Juan Miranda-Rios and Mario Soberón

Departamento de Microbiología Molecular. Instituto de Biotecnología, UNAM, Apdo Postal 510-3, Cuernavaca Morelos 62270, México

Most biosynthetic genes in bacteria are subject to negative feedback regulation by the end product of the metabolic pathway. This repression mechanism avoids unnecessary synthesis of enzymes saving energy and metabolic precursors. Feedback regulation needs a mechanism for sensing the concentration of the metabolite and

a mechanism for repressing gene expression. To date, two main mechanisms that repress gene expression have been documented, one at the level of transcription initiation and the second at the level of RNA translation and elongation. In all known cases, the sensing mechanism involves proteins that bind the effector molecule and either prevent transcription or RNA translation and elongation.

Thiamin pyrophosphate (TPP) is a cofactor of key enzymes of carbon metabolism. In *Rhizobium etli* we characterized a cluster of four genes named *thiCOGE* whose products are involved in TPP synthesis (1). Interestingly, the *thiCOGE* RNA contains an untranslated leader of 211 bases (1). Two RNA structures located in the RNA leader are important in regulating gene expression: a hairpin structure that overlaps the ribosome binding site near *thiC* initiating AUG, the second structure is called the '*thi box*' which is 38 bases length and highly conserved in many thiamin biosynthetic genes of very distinct bacterial species and archea (1, 2). We will discuss the possibility that the '*thi box*' directly senses thiamin concentration promoting the formation of the hairpin at the ribosome binding site preventing *thiC* translation and transcript elongation. The conservation of the '*thi box*' and the lack of a sensing protein suggest that the molecular mechanism involved in thiamin gene expression in bacteria represents an antique mechanism regulating gene expression in primitive ancestor organisms.

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ROUND TABLE IV GENOMES, EXTREMOPHILES AND EVOLUTION

THE COMPLICATED HISTORY OF A PROMISCUOUS OPERONJanet L. Siefert¹ and James F. Kasting²¹ *Department of Statistics, Rice University, Houston, Texas 77251;* ² *Geosciences Department, Penn State University, State College, PA 16802*

Microbial genome analysis has revealed that the architecture of the genome is quite fluid and dynamic. Limited operonic structures are found intact between closely related organisms. In fact, in spanning the phylogenetic breadth between archaea and bacteria less than 20 putative operons are found in common, the majority of these related to ribosomal function. The driving force behind the lack of conserved genome structure is likely to be horizontal gene transfer. This transfer of genetic material can be in pieces that may or may not include portions of genes, single genes, multiple genes, or operons. The presence and frequency of transfer is being indicted as a major reason for phylogenetic incongruity and the lack of confidence in single gene trees. We report the conservation of a unique putative operon whose phylogenetic history indicates multiple duplication events as well as a robust inclination to spread throughout the bacterial kingdom. The only consistent exceptions to this phenomenon are the absence of the operon in Archaea or cyanobacteria unless they have the capacity to fix nitrogen. This operon is composed of two genes, *nifS* and *nifU*, which are involved with iron-sulfur cluster formation. As iron-sulfur cluster formation is integral to the enzymes of many core metabolic functions of both Archaea and Bacteria, the first occurrence of this operon and its subsequent evolution are important. We report the phylogenetic reconstruction and whole genome analysis for 60 microbial genomes with respect to the following genes: *NifS* (2 paralogues), *NifU*, *IscA*, and *CobT*. We report the timing of two separate versions of this operon and discuss the likely evolutionary scenario that may have led to their phyletic distribution today.

IMPORTANCE OF CHEMOLITHOTROPHY FOR EARLY LIFE ON EARTH, THE TINTO RIVER CASEAmils, R.^{1,2}, Gómez, F.², González-Toril, E.¹, Fernández-Remolar, D.²¹ *Centro de Biología Molecular, U. Autónoma de Madrid, Cantoblanco, 28049 Madrid and* ² *Centro de Astrobiología, INTA-CSIC, Torrejón de Ardoz, 28850 Madrid, Spain*

An important number of acidic environments, pH lower than 3, are the product of the chemolithotrophic metabolism performed by prokaryotic microorganisms associated to the iron and sulfur cycles. The Tinto River (Iberian Pyritic Belt, Southwestern Spain) is an extreme environment with a constant acidic pH (mean 2.3), a high concentration of heavy metals (Fe, As, Cu, Zn, Cr, Ni) and a remarkable level of microbial diversity. The extreme conditions found in the river are the direct consequence of the active metabolism of chemolithotrophic microorganisms thriving in the rich polymetallic sulfides present in the ecosystem. Conventional and molecular ecology techniques were used to study the microbial ecology of the

Tinto system. Although sulfur metabolism plays an important role in the system, iron seems to be the key element in this habitat. Iron is not only an important electron donor, but also an electron acceptor for anaerobic respiration in the anoxic parts of the river. It is responsible for the maintenance of a constant acidic pH, probably critical for biodiversity, and for radiation protection. Laminar iron stromatolitic formations can be found along the river, These structures are related to massive laminated iron bioformations found at different elevations above the current river. A geomicrobiological model system of this ecosystem encompassing most of the geological, physical, chemical and biological variables will be presented and its implications for early life on our planet discussed.

ORIGIN OF DNA AND DNA GENOMES

Patrick Forterre

Institut de Génétique et Microbiologie, Université Paris-Sud, Centre d'Orsay, 91405 Orsay Cedex

Comparative genomics associated to molecular phylogenetic analyses have produced a wealth of new data during the last decade that can be tentatively used to reconstruct early cellular evolution. In particular, the problem of the origin of DNA and DNA replication systems is now in the middle of a hot debate. Although *Archaea* replicate their DNA according a bacterial mode, their replication machinery is typically eukaryotic (1). To explain the extreme divergence in the DNA replication mechanism of *Bacteria* on one side and *Archaea/Eukarya* on the other, it has been suggested that of the Last Universal Common Ancestor (LUCA) was still a member of the RNA world (2) or that a massive non orthologous displacement of DNA replication proteins occurred early on in *Bacteria* (3). Recent data and hypotheses have also highlighted the importance of the role of viruses in early cellular evolution, especially in the origin and evolution of DNA replication mechanism (3,4). I have suggested that DNA was invented as a modified form of RNA by viruses during the second age of the RNA world, and later on take over the role of RNA as cellular genetic material (5). New comparative genomic data indeed suggest that at least thymidine containing DNA has been invented twice and are in agreement with a viral origin.

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ACCESS TO EVOLUTION THROUGH THE GENOMIC SEQUENCING OF THREE HYPERTHERMOPHILIC ARCHAEA AND THROUGH THE DIRECT SEQUENCING OF ENVIRONMENTAL DNA

Yutaka Kawarabayasi

IMCB, National Institute of Advanced Industrial Science and Technology, AIST Tsukuba Central 6, Higashi 1-1, Tsukuba, Ibaraki, 305-8566 Japan (National Institute of Technology and Evaluation)

To identify a useful thermo-stable protein or enzyme, the genome analysis of the hyper-thermophilic archaea was performed. The first target was *Pyrococcus horikoshii*, an anaerobic euryarchaeon that was isolated from hydrothermal vent at Okinawa trough. The second was *Aeropyrum pernix*, an aerobic crenarchaeon that was isolated from Kodakara-jima islands. The third was *Sulfolobus tokodaii*, which is an aerobic and acidophilic crenarchaeon and was isolated from Beppu hot springs. The optimal growth temperature for *P. horikoshii* and *A. pernix* is over 90°C, that for *S. tokodaii* is about 80°C. The confirmed genome sizes are 1,738,505 bp, 1,669,695 bp and 2,694,756 bp, respectively. A total of 2061, 2694 and 2826 potential protein-coding regions (ORFs) were assigned, respectively. The function of 30% ORFs were estimated by similarity, 20% had similarity to hypothetical protein, and 7% has some motifs. The features specific to each species were extracted from these data.

Comparison of the entire genomic data among these three genomes provided the important information for evolution. From the result of comparison of RNA-coding genes and top-hit homologs, it was indicated that *P. horikoshii* and *A. pernix* were more similar than the present evolutionary position. The structure and organization of tRNA genes and other features indicated that *S. tokodaii* was very similar to Eukaryote.

To identify un-culturable organisms near the origin of life, direct sequencing of the environmental DNA were tested. The DNA directly prepared from ocean or hot springs was cloned into plasmid vector without amplification by PCR and each plasmid DNA was used for sequence analysis. These sequence data did not contain the sequence previously deposited into the database. These data provide the more important information for diversity of microbes than comparison of 16S rRNA sequences, I think. I also would like to discuss the possibility for identification of organism that survive from the period when the life was born.

ROUND TABLE V: LIFE IN THE ARCHEAN

THE OLDEST EVIDENCES OF LIFE

J. William Schopf¹, Andrew D. Czaja¹, Anatoliy B. Kudryavtsev², David G. Agresti³, Thomas J. Wdowiak³, André Kempe^{4,5}, Wladyslaw Altermann⁵, and Wolfgang M. Heckl⁴

¹ Department of Earth & Space Sciences, and Institute of Geophysics & Planetary Physics (Center for the Study of the Evolution and Origin of Life), University of California, Los Angeles, CA 90095-1567, USA; ² Astro and Solar System Physics Program, Department of Physics, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA; ³ Astro and Solar System Physics Program, Department of Physics, University of Alabama at Birmingham, Birmingham, AL 35294-1170, USA; ⁴ Institut für Kristallographie und Angewandte Mineralogie, Center for NanoScience, Ludwig-Maximilians-Universität München, D-80333 München, Germany; ⁵ Institut für Allgemeine und Angewandte Geologie, Ludwig-Maximilians-Universität München, D-80333 München, Germany

A minimum age for the antiquity of life is provided by the three oldest occurrences of cellular fossils now known, all petrified in carbonaceous cherts: from South Africa, unbranched microbial filaments of the ~3,375-Ma-old Kromberg Formation (Walsh & Lowe, 1985; Schopf *et al.*, 2002), and from Western Australia, ensheathed colonies of spheroidal cells from the ~3,430-Ma-old Strelley Pool Chert (Schopf & Packer, 1987) and unbranched filaments from the ~3,465-Ma-old Apex chert (Schopf, 1993; Schopf, 1994).

Although the biogenicity of the Apex microbes has recently been questioned (Brasier *et al.* 2002), our reexamination of the same specimens – by use of conventional and confocal microscopy and measurement of their composition by laser-Raman imagery backed by studies of microfossils in 23 other Precambrian cherts as well as atomic force microscopy of the submicron-scale wall structure of selected specimens – shows that they are unquestionably biological. All agree that the Apex filaments are carbonaceous (Walsh & Lowe, 1985; Brasier *et al.*, 2002) and that the carbon isotopic composition of the Apex kerogen falls well within the range established for Precambrian biological organic matter (Brasier *et al.*, 2002; Schopf, 1994; Strauss *et al.*, 1992), yet Brasier *et al.* hypothesize that the kerogen making up the Apex filaments is abiotic, formed ‘via Fischer-Tropsch-type synthesis’. No data are presented by these workers that permit identification of the hypothesized products; no mechanism is suggested for how such products came together to form sinuous cylindrical tapering segmented filaments. Not only are non-biologic carbonaceous structures of such complex morphology unknown in geological materials, no kerogens produced via Fischer-Tropsch-type synthesis (or by any other abiological process) have been identified in the Earth’s rock record.

The claim (Brasier *et al.*, 2002) that the Apex filaments are non-biological is incorrect. Rather, laser-Raman imagery of the fossils of the Apex, Kromberg, and Strelley Pool cherts shows a one-to-one correlation of organic composition and microbial morphology that confirms their biological origin. Together, these deposits provide a firm benchmark in the search for life’s antiquity: ‘By ~3,500 Myr ago, microbial life was flourishing and presumably widespread’ (Schopf *et al.*, 2002).

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ROUND TABLE VI: THE SEARCH FOR LIFE IN THE SOLAR SYSTEM

SEARCH FOR TRACES OF LIFE ON MARS

André Brack

Centre de Biophysique Moléculaire CNRS, Orléans, France (brack@cnrs-orleans.fr)

The search for traces of life on Mars encompasses a large number of disciplines (Brack *et al.*, 1999; Westall *et al.*, 2000). Beagle-2, the exobiology lander of ESA 2003 Mars Express mission, comprises an integrated suite of instruments to optimize the search for evidence of life on Mars in subsurface and rock interior samples. The package includes instruments to study sample mineralogy (composition, macroscopic and microscopic structural and textural features), organics (elemental, molecular), oxidation state and petrology (major and minor element composition). Environmental characteristics will also be measured (atmosphere, radiation budget, temperature, pressure, wind speed and direction, humidity, dust activity). The material will be sampled from protected sites (subsurface and rock interiors) with a mole and a small surface rock grinder and corer mounted on a robotic arm (Sims *et al.*, 1999). The solid sample (soil or rock) will be heated in steps of increasing temperature, each increment being supplied with freshly generated oxygen. Any carbon compound present will burn to give carbon dioxide. The gas generated at each temperature will be analysed by the mass spectrometer. The instrument can distinguish between the two stable isotopes of the carbon and quantify the ratio. Other gases can be analysed by the same instrument including methane.

The 'STONE' experiment, flown by ESA, was designed to test whether Martian sedimentary material could survive terrestrial atmospheric. A basalt (inflight control), a dolomite (sedimentary rock) and artificial Martian regolith were embedded into the ablative heat shield of Foton 12, which was launched on September 1999. The collected entry samples have been analysed for their chemistry, mineralogy and isotopic compositions by a European consortium. Modifications due to atmospheric infall were tested by reference to the untreated samples. The dolomite sample was retrieved intact, although reduced to a depth of about 30% of its original thickness, suggesting that some Martian sediments could, in part, survive terrestrial atmospheric entry from space. Some kinetic isotopic fractionation accompanied the thermal degradation of the dolomite during re-entry, as evidenced by bulk isotopic measurements on different zones of the residual carbonate. The silica 'fusion crust' from the associated sample holder exhibited a significant degree of isotopic exchange with atmospheric oxygen during re-entry (Brack *et al.*, 2002).

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FOSSIL MAGNETOTACTIC BACTERIA IN THE METEORITE ALH84001: THE EVIDENCE

E. Imre Friedmann

NASA Ames Research Center, Mail Code 245-3, Moffett Field, CA. (ifriedm@mail.arc.nasa.gov)

The evidence of fossil magnetotactic bacteria in the Martian meteorite ALH84001 will be summarized and presented, including projection of 3D electron micrographs.

The first line of evidence rests on the size, chemistry and unusual morphology of the magnetite crystals. Such crystals are known to be produced by magnetotactic bacteria but they do not occur as minerals (Thomas-Keprta *et al.*, 2001).

The second line of evidence rests on the presence of magnetite crystal chains in the meteorite, similar to those produced by terrestrial magnetotactic bacteria. Although small in size, these chains are biological structures of great complexity, cell organelles (magnetosome chains) built of magnetite crystals held together by organic components. Such *complex systems* can not be produced in nature by simple chemical and physical processes as they result from the interaction of a *number of processes*, including feedback loops. Six characteristics of such magnetosome chains, distinguishing them from abiotically produced magnetite crystal chains, have been identified. Using new microscopic methods, all these characteristics have been shown to be present ALH84001 (Friedmann *et al.*, 2001).

To date, no such chains could be demonstrated in bacterial magnetofossils described from terrestrial sites, due to the unavailability of suitable microscopic techniques. With the new methods now available, it can be shown that magnetite crystal chains in terrestrial bacterial fossils are highly similar to those described from ALH84001 (Friedmann, Wierzchos & Ascaso, unpubl.).

DOES THE RAPID APPEARANCE OF LIFE ON EARTH SUGGEST THAT LIFE IS COMMON IN THE UNIVERSE?

Charles H. Lineweaver, Tamara M. Davis

School of Physics, University of New South Wales, Sydney, NSW 2052, Australia (Astrobiology, 2002, in press)

It is sometimes assumed (e.g. Lineweaver, 2001) that the rapidity of biogenesis on Earth suggests that life is common in the Universe. Here we critically exam-

ine the assumptions inherent in this if-life-evolved-rapidly-life-must-be-common argument.

We use the observational constraints on the rapidity of biogenesis on Earth to infer the probability of biogenesis on terrestrial planets with the same unknown probability of biogenesis as the Earth.

We find that on such planets, older than ~ 1 Gyr, the probability of biogenesis is $>33\%$ at the 95% confidence level. This quantifies an important term in the Drake Equation but does not necessarily mean that life is common in the Universe.