Public Talk

GRAVITY IS COOL: OR, WHY OUR UNIVERSE IS HOSPITABLE TO LIFE

Freeman J. Dyson, Institute for Advanced Study, Princeton, New Jersey

The existence of life depends crucially on the fact that our universe has two faces, the quiet and the violent, cleanly separated from each other. The violent face, beginning with the big bang and continuing with supernova explosions, created the stuff that we are made of. The quiet face, symbolized by the harmony of the earth and the planets orbiting peacefully around the sun, sustains us and allows us to evolve. The fundamental reason why the two faces exist is the fact that the universe is dominated by the force of gravitation. Gravitational energy is quantitatively the largest reserve of energy, and qualitatively the least disordered. Because of its superior quality, gravitational energy can change easily and irreversibly into other forms of energy. Other forms of energy are associated with disorder and heat, but gravitational energy is cool. This is why gravitational energy can drive turbines in a hydroelectric power-station with almost a hundred percent efficiency, while other kinds of power-station, powered by coal or oil or natural gas or uranium, struggle to reach fifty percent. The gravitational energy of the ice in the high Canadian ice-cap during the last ice age waited quietly for a hundred thousand years, until a thaw and a break in an ice-dam released it. As soon as it was released, it changed into the turbulent energy of the flood that excavated a trillion tons of rock and created the channeled scablands of Washington State. All over the universe, when conditions are right for gravitational energy to be released, it can change instantly into heat and radiation, and a cataclysm results. The two faces of our universe are a consequence of the two faces of gravitation. Gravitation is the ordering principle that holds our earth together as a stage for us to walk on, and gravitation is the ultimate reservoir of energy that can smash our world to pieces.

i1.1

ATMOSPHERIC PREBIOTIC SYNTHESES

François Raulin
LISA, CNRS & Universités Paris 12 & 7, 94010 Créteil Cedex France

The formation of organic compounds that potentially play a role in prebiotic chemistry has been observed in a number of different atmospheres. This has been clearly demonstrated by three complementary approaches:

- experimental simulation in the laboratory, using model atmospheres, various energy sources and dedicated chemical reactors
- theoretical modeling, with the development, in particular, of 0-D to 3-D photochemical models
- direct observation of planetary atmospheres by remote sensing techniques or in situ analysis instrumentation's

Since the now historical experiment by Stanley Miller, laboratory simulations have shown that a model atmosphere submitted to energy deposition can yield a wide variety of organic compounds, including some of prebiotic interest, such as HCN or HCHO. For this to occur the atmosphere requires some reducing character, i.e. CH_4-NH_3 to CO-N_2, or even CO_2-N_2 -H_2 or CO_2-CH_4-N_2. The atmosphere which can yield the widest range of organic compounds appears to be a CH_4-N_2-H_2O gas mixture (Raulin and Frère, 1989). Furthermore, the organic material produced can be classified within two main categories : volatile organic compounds, and refractory products. The latter - now usually called « tholins » (Sagan and Khare, 1979), can yield many compounds of biological interest on hydrolysis. These solid products are thus of crucial prebiotic importance. However, they have not yet been systematically studied and their chemical properties (in particular molecular composition) as well as physical characteristics (in particular morphological and optical properties) are still not well known and must be determined (Coll et al., 1998).

Photochemical models generally confirm the results of simulation experiments. They show, in particular, that an oxidized CO_2 atmosphere can only produce organics in noticeable amounts if CO and/or H_2 are initially present. However, it must be emphasized that photochemical

models do not usually provide uncertainties with their data. This question also has to be considered.

Application of these data to the atmosphere of the primitive Earth strongly suggests that atmospheric processes may not have played a significant role as a source of organic matter in the primordial environment, since it is likely now that the primitive atmosphere of the Earth was oxidized. Nevertheless we have no direct information on the environmental conditions of the primitive Earth. Consequently, the observational approach in that case is more than difficult. However, the Solar system offers many examples of various atmospheres which can be considered to test our experimental as well as theoretical models.

Comparative planetology indeed confirms the results obtained from models (both experimental and theoretical). Organics have been found in the strongly (giant planets) or slightly (Titan) reducing planetary atmospheres and not in the oxidized atmospheres of Venus and mars. On the contrary, their presence now in the highly oxidized atmosphere of the Earth is a clear evidence for biological activity. Comparing the several different planetary atmospheres, among all the planets of the Solar system (Earth excepted), the CH_4-N_2 dense atmosphere of Titan appears as the richest atmosphere in organics, both in the gas and aerosol phases.

After a general review of atmospheric prebiotic-like syntheses, some of the most recent results obtained from observation, theoretical modeling and laboratory experiments related to these planetary objects will be presented and their implication to the field of the origin of life will be discussed.

Coll, P., Coscia, D., Gazeau, M.-C. and Raulin, F. : 1998, *Origins of Life & Evol. Biosphere* **28**, 195.
Lebreton, J.-P. : 1997, *Huygens Science Payload and Mission*, **ESA-SP1177**.
Raulin, F. and Frère, C. : 1989, *J. British Interplan. Soc.* **42**, 411.
Sagan, C. and Khare, B.N. : 1979, *Nature* **277**, 102.

i1.2

POLYHYDROXYLATED COMPOUNDS IN CARBONACEOUS METEORITES

George Cooper, Novelle Kimmich, and Katrina Brabham

NASA Ames Research Center, Moffett Field, CA 94035 USA

Carbonaceous meteorites contain numerous compounds of interest in studies of the origin of life and early solar system organic chemistry. These compounds include: amino acids, sulfonic acids, phosphonic acids, purines and pyrimidines (Cronin and Chang, 1993). Absent among the biologically important compounds reported in meteorites are sugars, polyhydroxy aldehydes or ketones (polyols). Ribose and deoxyribose, five carbon sugars, are central to the role of contemporary nucleic acids, DNA and RNA. Throughout the history of the solar system, meteorites and comets have delivered much organic matter to the Earth and other planets. If polyhydroxylated compounds are indigenous to meteorites they could have been part of the initial mixture of pre-biotic compounds that led to life on the early Earth.

One of the most generally agreed upon scenarios for natural abiotic synthesis of polyols is the "Formose" reaction (Langenbeck, 1956). In this reaction, formaldehyde, in aqueous solution, reacts with itself to gradually build a variety of compounds of increasing carbon number. Among the products seen are glycoaldehyde, ethylene glycol, glyceraldehyde, dihydroxyacetone, glycerol, erythrose, ribose, six-carbon sugars, etc. (Walker, 1964). Because there was aqueous alteration on the parent bodies of carbonaceous meteorites (Cronin and Chang, 1993), and formaldehyde is a ubiquitous interstellar molecule, the Formose reaction would have been possible in meteorites. Among the techniques used in the search for meteoritic polyols are gas chromatography-mass spectrometry (GC-MS) and gas chromatography-isotope ratio mass spectrometry (GC-IRMS). Isotope ratio measurements, especially D/H, and $^{13}C/^{12}C$, have been used to show the extraterrestrial nature of meteoritic organic compounds (Cronin and Chang, 1993).

A GC-MS examination of two carbonaceous meteorites, Murchison and Murray, known to contain many soluble organic compounds, revealed a series of polyols (sugar alcohols) that was apparently formed by an abiotic mechanism. This series extends through at least the four-carbon members. Bulk isotope measurements of Murchison polyols show that the majority are indigenous to the meteorite. However, because of the possibility of Earthly contamination by widespread compounds such as glycerol, ethylene glycol, etc., measurement of individual compounds by GC-IRMS is a necessity. Results of GC-IRMS and molecular analysis will be presented.

Cronin, J. R. and Chang, S. (1993) *In The Chemistry of Life's Origin*, p. 209-258, Eds. J. M. Greenberg et al., Kluwer Academic Publishers, The Netherlands.

Langenbeck, W. (1956) J. Prakt. Chem. (**4**) **3**, 196-210.

Walker, J. F. (1964) *Formaldehyde*. Reinhold Publishing Corp.

i1.3

THE COORDINATION-CHEMICAL FOUNDATION OF LIFE

Gunter Wachtershauser
Tal 229, D-80331 Munich

A methodology of functional retrodiction from the last common ancestor towards the origin of life involves the construction of common precursor functions for disparate evolutionary successor functions. Three examples for this methodology are given:

(1) The retrodiction of redox bio-energy, carbanion bio-energy, group activation and chemi-osmosis into a primordial redox energy source of the formation of pyrite (FeS_2) from FeS and H_2S;

(2) The retrodiction of cell envelopes, enzyme surfaces, iron-sulfur clusters, thermo-stable enzyme folding and the metabolism of anionic constituents, notably of phosphorylated sugars and polyanionic peptides into a two-dimensionally ordered surface metabolism on positively charged pyrite surfaces with surface-bonded iron-sulfur clusters.

(3) Metabolic pathways are retrodicted into an archaic core metabolism consisting of a feeder pathway, akin to the extant reductive acetyl-CoA pathway; a carbon fixating antocatalytic reproduction cycle, akin to the extant redutive citric acid cycle and branch pathways radiating therefrom with products exhibiting dual feedback: into the core metabolism and into their own branch pathways.

Experimental results will be reported which demonstrate the key reaction in the archaic feeder pathway: the formation of activated acetic acid; a key reaction in the archaic feeder pathway: the formation of activated acetic acid; a key reaction in the archaic reproduction cycle: the energy-conserving reduction of keto acids; and a key reaction in the branch pathways: the formation of amino acids by reductive amination. For all these bio-organic reactions the presence of FeS is mandatory. Therefore, the original homestead for this chemo-autotrophic origin of life is an "iron-sulfur world" in an environment of magmatic exhalations.

c1.4

BORON COMPOUNDS IN THE PRIMITIVE EARTH. IMPLICATIONS FOR PREBIOTIC EVOLUTION

Romulus Scorei, Gyury Steinbrecher, Vily Marius Cimpoiasu, Iulian Petrisor, Vilma Scorei, Mihaela Mitrut

University of Craiova, A.I.Cuza 13,1100 Craiova, ROMANIA

In spite of its low natural abundance, B is widely distributed in both the lithosphere and hydrosphere. In rocks its concentrations averages are about 10-20 mg $B \cdot kg^{-1}$. In sea water it can range from ca. 1-10 mg $B \cdot kg^{-1}$, while its concentration in river water is about 1/350 that of sea water. The B level in animal tissue average ca. 0.3 mg $B \cdot kg^{-1}$ on a dry wt basis with a range of 0.03-0.4 mg $B \cdot kg^{-1}$ in blood and as high as 1.2 mg $B \cdot kg^{-1}$ in areas with a high B content in the water supply.

Boron is found in erythrocytes, blood serum and plasma and at about 0.01-0.1 mg $B \cdot kg^{-1}$ and in plants at 10-100 mg $B \cdot kg^{-1}$ dry wt. Naturally occurring B is found exclusively bound to oxygen as borates, less often as boric acid or, much more rarely, to fluorine as in the BF_4^- ion.

High-temperature origin of life theories requires that the components of the first genetic material are stable. It is known that one of the most stable borate esters is formed with "cis-diol" on a furanose ring. The presence of the boric acid in the planetary prebiotic environment was mainly due to the volcanic activity, which has a particular ability to form stable complexes with compounds having "cis-diol" groups.

We have established that the decomposition rate of the D-glucose and D-ribose in the presence of boron (at pH 8) is low compared with the same sugars without boron, but after the appearance of the reaction products, they undergo a complexing process with boron and at high temperature there takes place an obstruction of the open-chain form and an almost entire slowing down of the decomposition of the beta-anomer form. The obstruction of the decomposition of the monosaccharides in the basic medium by the boron complexed reaction products is a proof of using as prebiotic reagents under special conditions (high temperature).

c1.5

CHEMICAL REDUCTION AND ACTIVATION OF PHOSPHATE ON THE PRIMITIVE EARTH

Dietmar Glindemann,[1] Rob M. De Graaf,[2] and Alan W. Schwartz [2]
[1] University of British Columbia, Dept. of Chemistry, 2036 Main Mall, Vancouver, BC, V6T 1Z1, Canada. [2] Evolutionary Biology Research Group, Faculty of Science, University of Nijmegen, Postbus 9010, 6500 GL Nijmegen, The Netherlands.

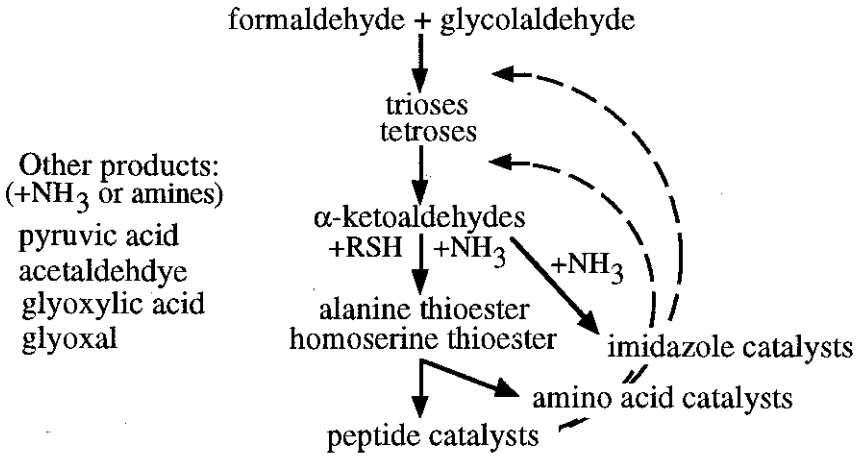
If phosphorus played a role in the origin of life on Earth, some means of concentrating the micromolar levels of phosphate which at present occur in natural bodies of water must first have been available. While the possible presence on the primitive Earth of lower oxyacids of phosphorus - which have water soluble calcium salts - would offer a solution to this problem, only meteoritic schreibersite or a large-scale reductive event during early core formation have been suggested as possible sources for such species. We now show, however, that simulated (mini)lightning discharges in model prebiotic atmospheres are capable of reducing orthophosphates, including apatite. The reduction is surprisingly efficient, even under only weakly reducing conditions, and produces phosphite in substantial yield. We argue that the occurrence of lightning in volcanic eruption clouds, which have been suggested previously as uniquely favorable sites for prebiotic syntheses, would have provided a mechanism for phosphite synthesis on the prebiotic Earth. Phosphite, which could have become concentrated by evaporation in volcanic areas, reacts thermally with nucleosides to produce nucleoside phosphites, and thereby provides a possible route for the synthesis of the first nucleotides.

c1.6

FORMATION OF AMINO ACID THIOESTERS FOR PREBIOTIC PEPTIDE SYNTHESIS: CATALYSIS BY AMINO ACID PRODUCTS

Arthur L. Weber
SETI Institute, Ames Research Center, MS 239-4, Moffett Field, CA 94035

The origin of life can be described as a series of events in which a prebiotic chemical process came increasingly under the control of its catalytic products. In our search for this prebiotic process that yielded catalytic take-over products (such as polypeptides), we have been investigating a reaction system that generates peptide-forming amino acid thioesters from formaldehyde, glycolaldehyde, and ammonia in the presence of thiols (Weber 1998). As shown below, this model process begins by aldol condensation of formaldehyde and glycolaldehyde to give trioses and tetroses. These sugars then undergo beta-dehydration yielding their respective α-ketoaldehydes. Addition of ammonia to the α-ketoaldehydes yields imines which can either (a) rearrange in the presence of thiols to give amino acid thioesters or (b) react with another molecule of aldehyde to give imidazoles (Kort 1970). This 'one-pot' reaction system operates under mild aqueous conditions, and like modern amino acid biosynthesis, uses sugar intermediates which are converted to products by energy-yielding redox reactions (Weber 1997). Recently, we discovered that amino acids, such as the alanine reaction product, catalyze the first and second steps of the process. In the presence of ammonia the process also generates other synthetically useful products, like the important biochemical -- pyruvic acid.



Weber, A. L.: 1998, Origins of Life **28**, 259-270.
Weber, A. L.: 1997, J. Mol. Evol. **44**,354-360.
Kort, M. J.: 1970, Adv. Carbohydr. Chem. Biochem. **25**, 311-349.

c1.7

LIPOSOME-ASSISTED SELECTIVE POLYCONDENSATION OF α-AMINO ACIDS AND PEPTIDES

Markus Blocher, Daojun Liu, and Pier Luigi Luisi
Institute for Polymers, Department of Material Sciences, ETH Zürich, Switzerland

One of the unsolved problems in the prebiotic chemistry concerns the origin of functional polypeptides. Our work deals with this topic and with the question, whether lipidic bilayers can aid the polycondensation of amino acids and short peptides.

An investigation of the selectivity effects induced by 1-palmitoyl-2-oleoyl-*sn*-glycero-3- phosphocholine liposomes on the polycondensation of hydrophobic α-amino acids and peptides is presented. Two types of reactions are studied: (i) The polymerization of N-carboxy anhydride (NCA) amino acids (i. e., NCA-Trp), and (ii) the polycondensation of dipeptides in the presence of the hydrophobic condensing agent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ).

In case (i), the condensation of hydrophobic NCA-amino acids, much longer oligomers (up to 29mer) can be obtained in the presence of liposomes, whereas in the aqueous solution without liposomes the maximal length was around the 7mer. In the case (ii), the membrane-aided polycondensation of dipeptides, such as H-Trp-Trp-OH, led also to higher oligomers (up to H-Trp_8- OH), whereas in the aqueous control experiment only traces of H-Trp_4-OH were found.

If the liposomes were exposed to a small library of four different dipeptides (H-Trp-Trp-OH, H- Trp-Gly-OH, H-Trp-Asp-OH, H-Trp-Glu-OH), only the most hydrophobic H-Trp-Trp-OH was selected by the membrane and underwent oligomerization. Out of the theoretical 16 possible tetrapeptides, H-Trp_4-OH makes about 70% of all the tetrapeptides formed.

Charged membranes can also bind opposite charged amino acids or peptides on the basis of electrostatic interactions. This allows polycondensations on hydrophobic and electrostatic interactions. This can in principle lead to the formation of polypeptide chains consisting of different types of amino acids, which are a prerequisite for obtaining polypeptides with functionality.

P1.1

DEPHOSPHORYLATING ACTIVITY OF RARE EARTH ELEMENTS AND ITS IMPLICATION IN THE CHEMICAL EVOLUTION

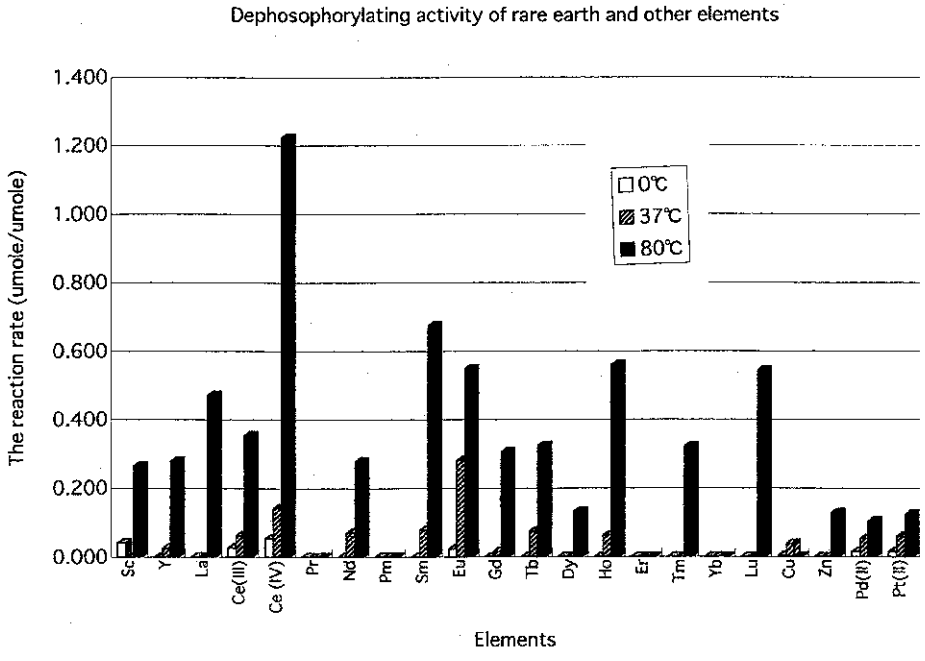
Mitsuhiko Akaboshi, Kenichi Kawai, Yoshiko Tanaka, Keizo Kawamoto and Noriko Fujii

Research Reactor Institute, Kyoto University, Kumatori-cho, Sennan-gun, Osaka 590-0490, Japan

Recently, significant interest has been focusing onto the hydrolysis of DNA, RNA[1] and 3',5'-cyclic adenosine monophosphate[2] by rare earth elements (REEs). We also found a strong dephosphorylating activity of REEs using p-nitrophenyl phosphate as a substrate[3]. Present report is concerned with the effect of physical and chemical factors on the dephosphorylating activity of REEs, and further the implication of these activities in the chemical evolution.

METHODS

Catalytic avtivity of REEs to cleave phosphate ester bond was measured using alkaline phosphatase activity assay kit (Kirkegaard & Perry Laboratories Inc.).



P1.1 continued

RESULTS AND DISCUSSION

Fig. shows the dephosphorylating activities of REEs and other elements at the different temperatures.Among all the elements examined, the activity of Ce(IV) was the most highest, and the activities of Sm and Ho followed Ce(IV). Under the ordinary condition (37-℃,1 hr), It could hydrolyze 0.14 μmole of p-nitrophenyl phospahates per 1 μmole Ce(IV). The magnitude of this activity was about $1/1.93 \times 10^5$ that of standard alkaline phosphatase. It was also found that the phosphate liberating activity of Ce(IV) increased exponentially with increasing temperature until at least 80 ℃, while that of alkaline phosphatase completely fall down at that temperature. Moreover, it was found also that the activity did not change at different pH, while that of the enzyme was completely missing at pH 5.4.

From these experimental facts and the findings that considerably higher concentration of REE might be dissolved in primordial sea water[4], It can be easily presumed that accumulation of monoester phosphate compounds, such as AMP, GMP etc. the concentration of which in the sea water were higher enough to produce nucleic acids in the later process of the chemical evolution might be impossible. This leads to the conlusion that the origin of life as a consequence of chemical evolution might also be impossible. However, it is a universal fact that lives are on the present earth, and they have come here through the chemical evolution. This let us reach to a working hypothesis that there might have been some mechanism to supress the dephosphorylating activity of REEs in the primitive ocean. In order to confirm this hypothesis, search was made for some substance which have an inhibitory effect to the dephosphorylating activity of REEs. We found that some proteins such as albumin, histone and gelatin showed a strong inhibitory effect but not amino acids, bases and starch. The implication of these obsevations for the chemical evolution will be discussed.

References

1) Komiyama M. and Sumaoka J; *Curr. Opin. Chem. Biol.*, 2, 751-757 (1998).
2) Sumaoka J., Yashiro M. and Komiyama M; *Nucleic Acids Symp Ser.* 27, 37-38 (1992).
3) Akaboshi M.,Kawai K., Kawamoto K., Takada J., and Fujii N; *Viva Origino*, 27 (1999).
4) German C. R., Masuzawa T., Greaves M. J., Elderfield H. and Edmond J. M., *Geochim. Geophys. Acta,* 59, 1551-1558 (1955).

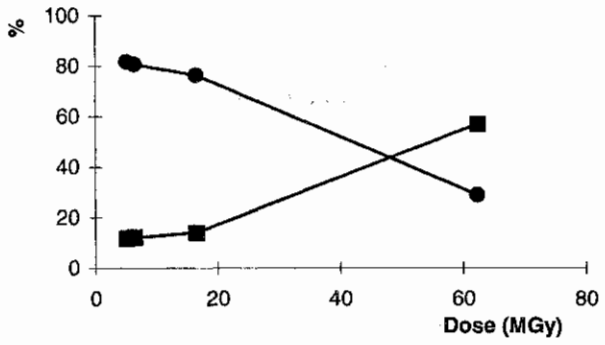
P1.2

RADIOLYTIC PRODUCT DISTRIBUTIONS FROM SELF-IRRADIATED SOLID STATE $Ca^{14}CO_3$

G. Albarrán[1], K.E. Collins[2], C.H. Collins[2]
[1] Instituto de Ciencias Nucleares, UNAM, Apdo. Post. 70-543, Coyoacan, 04510 Mexico D.F., Mexico; E-mail: albarran@nuclecu.unam.mx
[2] Instituto de Química, UNICAMP, Caixa Postal 6154, 13083-970 Campinas, SP, Brazil., E-mail: chc@iqm.unicamp.br

The study of prebiotic chemistry has focused on the chemical evolution of compounds containing the most important biogenic elements: C, H, O and N. Relevant to this study is the reduction of carbonates, compounds containing carbon in its most oxidized form, as a result of the radiation arising from the disintegration of radionuclides such as potasium-40, uranium-238, thorium 232, etc. present on the Prebiotic Earth. This radiation produces several radicals, principally CO_2^-, which have been observed by ESR and other analytical methods. These radicals produce secondary species in the solid which could then result in the products observed upon dissolution.

Dissolution of self-radiolyzed solid $Ca^{14}CO_3$ produces at least six carboxylic acids, formaldehyde and methanol. A comparison between the percent quantities of formic (●) and oxalic (■) acids after different self-



irradiation doses are shown in the Figure. The number of acetic, glyoxylic and glycolic acid molecules formed also increases as the dose increases, although the percent distributions do not change significantly and the initial G values are similar.

Thus, when the quantity of the CO_2^- ion-radical and the other radicals formed in the solid increase, the combination of these species produces changes in the distribution of the radiolytic products and the formation of secondary products, including malonic acid, which is also obtained in the radiolysis of solid formic acid.

CONACyT, CNPq, FAPESP, FAEP-UNICAMP

P1.4

ELECTRIC DISCHARGES AND THE ORIGIN OF LIFE: A HISTORICAL REASSESSMENT OF EARLY EXPERIMENTAL WORK

Jeffrey L. Bada[1], Gerhard Kminek[1] and Antonio Lazcano[2]
[1]Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0212;
[2]Facultad de Ciencias, UNAM/Apdo. Postal 70-407, Cd. Universitaria, 04510 Mexico D. F., MEXICO

One of the earliest and most well-known statements linking electricity with the origin of life is Charles Darwin's famous remark about a warm little pond in which "with all sorts of ammonia and phosphoric salts, light, heat, electricity present, that a protein compound was chemically formed". During the 19th century a large amount of research on electric discharges with various gas mixtures were carried out which led to the synthesis of fatty acids, sugars, and hydrogen cyanide (Glockler and Lind, 1939). But none of these 19th century experiments apparently led to the formation of amino acids. These experiments were mainly designed to understand the mechanism of photosynthesis, and did not address the issue of organic syntheses in a prebiotic context or the origin of life itself. Some of these observations found their way into Oparin's and Haldane's theories on the appearance of life.

In the first part of this century, Walther Löb reported the synthesis of glycine by exposing wet formamide to a silent discharge (Löb, 1913). Löb suggested that because of either the UV-light or the electrical field generated by the silent discharge, formamide was first converted to oxamic acid which in turn was reduced to glycine. In addition, Löb claimed that glycine was also produced when wet carbon monoxide and ammonia were subjected to the silent discharge and he proposed formamide as the intermediate in this synthesis. Lob theorized that glycine might also be produced from wet carbon dioxide and ammonia in a pathway wherein formamide was again the intermediate, however, he did not directly demonstrate this. Although Löb apparently did produce glycine from formamide this can not be considered a prebiotic reaction because formamide would not have been present on the primitive Earth in any significant concentrations. It is also possible in his experiments with wet carbon monoxide and ammonia that Löb synthesized HCN, which upon polymerization and hydrolysis gave glycine. To the best of our knowledge, Löb's work was first discussed within the context of prebiotic chemistry by Miller (1955).

P1.4 continued

Recently, Yockey (1997), Wächtershäuer (1998) and Arrhenius (Mojzsis et al., 1999) have claimed that Miller (1953) was not the first to carry out a prebiotic synthesis of amino acids using a spark discharge, which they credit to Löb. They have began a campaign to elevate Löb's work, with Yockey in particular stating that "Löb's priority must be recognized". From a careful reading of Löb's 1913 paper (in turn of the century German!) it is clear that his motivation for doing the experiment was to try to understand the assimilation of carbon dioxide and nitrogen in plants. There is no indication that he had any interest or even awareness in the question of how life began on Earth, or in the synthesis of organic compounds under possible prebiotic conditions. Neither Oparin, Haldane or Urey made any mention of Löb's work, which given Oparin's extensive review of early relevant literature suggests it was considered unimportant. Although some of Löb's results may have some bearing on our understanding of prebiotic syntheses, part of the significance of Miller's (1953) classical experiment lies not only in the production of amino acids and and other compounds, but in their synthesis under what was viewed at the time as plausible primitive Earth conditions.

References

Glocker, G. and Lind, S. C. (1939) "The Electrochemistry of Gases and other Dielectrics" (Wiley and Sons, Inc., New York).
Löb, W. (1913) Ber. 46: 684.
Miller, S. L. (1953) Science 117: 528.
Miller, S. L. (1955) JACS 77: 2351.
Mojzsis, S. J., Krishnamurthy, R., and Arrhenius, G. (1999) In Gesteland, R., Cech, T., and Atkins, J. (eds) "The RNA World II" (Cold Spring Harbor, New York), 1.
Wächtershäuer, G. (1998) In Brack, A. (ed.) "The Molecular Origins of Life" (Cambridge University Press, Cambridge) 206.
Yockey, H. P. (1997) Persp. Biol. Med. 41: 125.

P1.5

PREBIOTIC PEPTIDE CHEMISTRY ON MINERAL SURFACES

Bernard Barbier, Marylène Bertrand, Fabrice Fleury and André Brack
Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, 45071 Orléans cedex 02, France (brack@cnrs-orleans.fr).

Micrometeorites probably delivered significant amounts of organic molecules to the primitive Earth (Maurette, 1998). They are related to CM chondrites, a family of carbonaceous meteorite represented by the Murchison meteorite. The interplanetary grains contain also a high proportion of metallic sulfides, oxides and clays which are known to exhibit catalytic properties. The grains may therefore have functioned as tiny chondritic chemical reactors when reaching oceanic water.

We have studied the influence of different minerals (cristalline CdS, metallic molybdenum and montmorillonite clay) on the condensation of thioesters of amino acids present in the Murchison meteorite and on the self-organization of polypeptides based on the Murchison amino acids.

Leucine thioethyl ester condenses to dileucine, trileucine and leucine diketopiperazine in the presence of cadmium sulfide, metallic molybdenum or montmorillonite. Leucine and dileucine thioesters adsorb onto the mineral surfaces. Dileucine thioethyl ester condenses to tetraleucine and to diketopiperazine.

Strictly alternating poly(Glu-Leu) exhibits a random coil conformation in water because of charge repulsions (Bertrand and Brack, 1997). In the presence of a suspension of 100 equivalents of cristalline CdS (1-3μm), the polypeptide self-organizes into water soluble beta-pleeted sheets. By ageing or at higher CdS concentrations, the polypeptide precipitates. Peptide precipitation occurs at lower CdS concentrations when the sulfide is grinded suggesting that the polypeptide adsorbs on fine grains. Adsorption decreases when shortening the chain length. In the presence of a suspension of 50 equivalents of metallic molybdenum (3-4 μm), the polypeptide adopts an alpha-helical conformation. At higher molybdenum concentrations, the polypeptide precipitates. When adding increasing amounts of montmorillonite clay to the polypeptide in aqueous solution, the polypeptide is progressively adsorbed and does not self-organize in solution.

Bertrand, M. and Brack, A. :1997, Origins Life Evol. Biosphere **27,** 585-595.
Maurette, M. :1998, Origins Life Evol. Biosphere, **28**, 385-412.

P1.6

IMPROVING RELIABILITY OF COMPOUND IDENTIFICATION IN ABIOTIC SYNTHESIS SIMULATIONS: "TITAN COMPOUNDS"

V.A. Basiuk[1], Y. Benilan[2], R. Navarro-González[1] and F. Raulin[2]
[1] Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México, Circuito Exterior C.U., 04510 México, D.F., MEXICO;
[2] LISA, UMR CNRS 7583, Universites Paris 12 & Paris7, Avenue du General de Gaulle F-94010, Creteil Cedex, FRANCE

Gas chromatography/Fourier transform IR spectroscopy/mass spectrometry (GC/FTIR/MS) is one of the most powerful tools for the separation and unambiguous identification of complex mixtures of organic compounds. The two kinds of spectra drastically increase identification reliability when spectral databases are used; but there are some cases where the IR and mass spectra cannot be found in the databases. Sometimes this situation can be resolved by running standards; but this works only if one has more or less exact idea on the composition of analyzed unknowns, and such standards are indeed available. What to do if this is not the case?

Some studies related to the origins of life and abiotic formation of organic compounds can be a typical example when one encounters with a whole spectrum of analytical problems of this sort, hard or impossible to resolve in traditional ways: e.g., one cannot predict all possible compounds formed, some of them are unstable, unavailable as standards, or at worst have never been synthesized and spectrally characterized. During the GC/FTIR/MS identification of amino acid pyrolysis products we systematically faced the latter case, and the only solution found was to involve semi-empirical IR spectra simulations to compare the calculated spectra to experimental ones. The reasons why just semi-empirical methods, and not ab initio ones have been used are as follows: (1) running the ab initio calculations on powerful computers turns to be very expensive; (2) pyrolytic experiments similar to those performed with amino acids, can produce tens of compounds for which FTIR and mass spectra can be recorded. For relatively complex molecules, especially for those having several similar functional groups or substituents, pyrolytic losses can produce several possible isomers to

be considered for the IR spectra simulations. On the other hand, the calculations (first energy minimization and then IR spectrum simulation itself) for a compound of more than 250 dalton-molecular weight take normally more than 2 days. Thus, while the experimental procedures of pyrolysis, sample preparation and running one chromatogram with spectra acquisition take in total a few hours, subsequent compound identification involving auxiliary procedure of the IR spectra simulation extends to several weeks. Under such circumstances, we had no interest and extra time for detailed treatment of the simulated spectra (normal coordinate analysis, scaling, etc.). What was of real help instead is a general view of the latter and how good they match a general view of the experimental counterparts.

Can this approach be of more general use, that is for the GC/FTIR/MS identification of other types of compounds? One can point to at least one more area where this approach would be indispensable: laboratory simulation of organic compound formation in planetary and satellite atmospheres. Classic example is electric discharge simulations for chemical processes in the atmosphere of Titan, where a tremendous number of organic compounds is formed. Among the most important products are nitriles: due to their abundance as primary products, intense pyrolytic formation from higher-molecular-weight products, and crucial role in the pathways which might lead to compounds of biological importance, e.g. amino acids. Although a significant number of nitriles has been already identified (with the use of spectral databases), a lot of chromatographic peaks correspond to compounds unknown so far. Branched and unsaturated nitriles are very likely candidates for the latter, and for many important representatives, spectral data and standards are unavailable, making GC/FTIR/MS with auxiliary simulation of IR spectra the only remaining way of identification.

The main goal of the present study was to estimate applicability to cyanoacetylenes and other compounds of interest for Titan's atmospheric chemistry, of four semi-empirical methods (PM3, AM1, MNDO and MINDO3), which gave reasonable results for cyclic amides.

P1.7

Proposed in-situ hydrothermal condensation reactions of biologically relevant molecules on catalytic pyrite and smectite

María Catalina, Departments of Biochemistry* and Astrophysics†
University of California Santa Cruz, Santa Cruz, CA 95064

What environments on the present-day Earth could serve as model sites for testing plausible synthetic condensation reactions that could have led to functional biomolecules? This poster poses the question: Could a metal sulfide complex in a dilute prebiotic broth act as the primary catalyzing agent for biologically relevant molecules, as suggested by Huber and Wächtershäuser's, and can catalytic clay surfaces promote early condensation products, as suggested by J.P. Ferris, resulting in the oligomerization of nucleotides?

Consideration of energy sources, energy pathways, and environmental constraints are critical aspects in the understanding of how to investigate the events leading to the origin of life. Based on hydrochemical and thermodynamic properties of mineral metamorphosis in geothermally active areas, one such model site would be the caldera of Yellowstone National Park, Wyoming, USA.

In this case, we look to the caldera for the extreme environments, fit only for extremophiles, as a prebiotic analogue of the early Earth in support of a hyperthermal theory for the origin of life. A model is presented for testing Huber and Wächtershäuser's "theory of a pyrite-pulled chemoautotrophic origin of life" with admixtures of metal sulfide complex in the presence of smectite clay in-situ at a siliceous class of goethermal spring.

Methods of analysis will include quantitative measurement of structural moieties of modified nucleosides using liquid chromatography-mass spectrometry, elemental analysis using inductively coupled plasma mass spectrometer, images of macromolecular surface morphologies using Scanning Electron Microscopy , and characterize atomic morphologies using an integrated technique of light microscopy with tapping Atomic Force Microscopy in a Dvorak-Stotler controlled-environment chamber.

Expected results of the experiments are to obtain evidence that will determine whether the proposed reaction pathways can occur in a under natural conditions.

Arrhenius, G.: 1997, Origins of life and Evolution of the Biosphere **27**, 485-503
Deamer, D.W.: 1997, Microbiology & Molecular Biology Reviews **61**, 239-261
Huber, C. and Wächtershäuser, G.: 1998, Science **281**, 670-672
Ferris, J.P.: 1996, Nature **381**, 59-61
Nagao, E. and Dvorak, J.A.: 1997, Journal of Microscopy **191**, 8-19

María Catalina, e-mail: m_cat@cats.ucsc.edu
Mentors: *David Deamer, Emer., Dept. of Chemistry/Biochemistry UCSC
Sherry Cady, SETI Institute and NASA Ames Research Center;
†Frank Drake, SETI Institute & Dept. of Astronomy/Astrophysics UCSC
Gustaf Arrhenius, Scripps Institution of Oceanography

P1.8

THE INSOLUBLE HCN POLYMER IS A PREBIOTIC SOURCE OF ADENINE

Eduardo Borquez and Stanley Miller
Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0506 USA smiller@ucsd.edu

Oró (1) discovered the prebiotic synthesis of adenine from the polymerization of concentrated NH_4CN solutions and investigated the reaction in considerable detail (2, 3). Variations on the synthesis have been carried out using a photochemical step (4) and by using formaldehyde as a catalyst (5).

The Oró polymerization is usually carried out at 80-100 C, but it is not clear whether it will produce adenine at lower temperatures. We therefore measured the yield after long periods of reaction at temperatures between -80 and +100°C. Preliminary results indicate that the adenine yield is approximately independent of temperature. This suggests that the frozen earth model of prebiotic synthesis would be more efficient than elevated temperature in the prebiotic synthesis of adenine since it is much easier to concentrate NH_4CN by freezing than at elevated temperatures.

Oró and Kimball (2) also showed that the 6N HCl hydrolysis of the NH_4CN polymerization supernatant greatly increased the adenine yield. However, this hydrolysis also decomposes the adenine. Therefore, we measured the yields from an HCN polymerization mixture as a function of hydrolysis time and find that shorter hydrolysis periods give higher yields. We also investigated the hydrolysis at pH 8 of the supernatant, which is a better prebiotic model, and find that the adenine yield is comparable to the acid hydrolysis yield (~0.1%). There is no drop in yield at long hydrolysis times because of the stability of adenine at pH 8.

The insoluble black polymer formed from the NH_4CN reaction has been analyzed for amino acid content (6,7) but not as a source of adenine. The polymer was analyzed by both acid and neutral hydrolysis giving adenine yields in the order of 0.05%. This shows that the polymer may have been as important a prebiotic source of adenine as the usually analyzed supernatant. These results show that the NH_4CN polymerization reaction is a more efficient prebiotic source of adenine than previously thought.

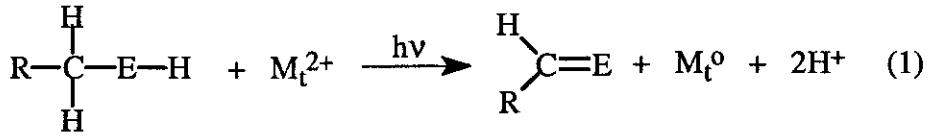
1. Oró, J. (1960) Biochem. Biophys. Res. Commun. 2, 407-412
2. Oró, J., Kimball A. P. (1961) Arch. Biochem. Biophys. 94, 217-227.
3. Oró, J., Kimball A. P. (1962) Arch. Biochem. Biophys. 96, 293-313.
4. Sanchez, R. A., Ferris, J. P., Orgel, L. E. (1967) J. Mol. Biol. 30, 223-253.
5. Schwartz, A. W., Goverde, M. (1982) J. Mol. Evol. 18, 351-353.
6. Ferris, J. P., Donner, D. B., Lobo, A. P (1973) J. Mol. Biol. 74, 499-510.
7. Matthews, C. N., (1992) Orig. of Life Evol. Biosphere 21, 421-434.

P1.9

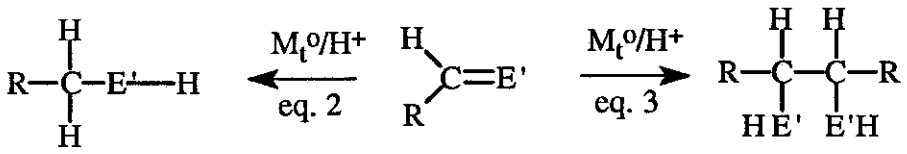
PHOTOCHEMICAL TRANSITION METAL-MEDIATED REACTIONS IN PREBIOTIC SYNTHESIS

John J. Eisch and Stanley L. Miller
State University of New York at Binghamton, Binghamton, NY, USA, 13902-6016 and University of California at San Diego, NSCORT/Exobiology, La Jolla, CA 92093-0506, USA

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 Cu, Ni, Co, and Fe offer a novel and versatile approach for harnessing 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 carbohydrates.



E = O, NH, CH_2 M_t = Cu, Ni, Co, Fe



Precedents for the photochemical and thermal reactions of transition metals depicted in eqs. 1-3 are known (Eisch et al., 1994, 1997, 1998). The pertinence and utility of these processes in prebiotic organic synthesis will be delineated in the presentation.

Eisch, J.J.,Shah, J.H.and Boleslawski, M.P.: 1994, J. Organomet. Chem., 464, 11.
Eisch, J.J., Shi, X., Alila, J.R. and Thiele, S.:1997, Chem. Ber./Recueil, 130, 1175.
Eisch, J.J., Aradi, A.A., Lucarelli, M.A. and Qian, Y. 1998, Tetrahedron, 54, 1169.

P1.10

EQUILIBRIUM AND KINETICS OF THE ALDOL CONDENSATION OF CYANOACETALDEHYDE

Carles Estévez* and Stanley L. Miller
Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, CA 92093-0506. USA. smiller@ucsd.edu
(*) Present address: Chemistry Department. Institut Universitari de Ciència i Tecnologia. Alvarez de Castro, 63. 08100 Mollet del Vallès. Barcelona, Spain.

It has been shown that cyanoacetaldehyde (CA) is a precursor to pyrimidines. Ferris *et al.* (1974) first showed that CA (10^{-3} to 10^{-1} M) reacts with guanidine to produce 2,4-diaminopyrimidine, which is a source of cytosine, isocytosine and uracil by hydrolysis. The yields ranged from 0.1 to 2%. However, there was no detectable cytosine (or uracil) produced from cyanoacetaldehyde and dilute urea solution.

Recently, Robertson and Miller (1995) showed that CA reacts with concentrated solutions of urea to form cytosine in yields of 30-50%, from which uracil can be formed by hydrolysis. Also, Robertson *et al.* (1996) reported the efficient synthesis of 2,4-diaminopyrimidine from CA and concentrated solutions of guanidine with yields as high as 85%. In both cases, CA dimerization was a competing reaction to pyrimidine formation and CA decomoposition. This has been proposed to explain the lower yields obtained when low concentrations of urea and guanidine are used.

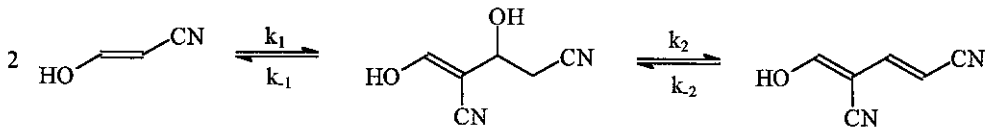
We have investigated the aldol self-condensation of CA since it is an important competing reaction for pyrimidine synthesis. The aldol condensation of CA is a consecutive two-step reversible reaction. In the first step CA undergoes a self condensation to give the ketol Y. The forward rates have been examined previously by Raulin and Toupance (1975). The rate of CA condensation was found to be proportional to the square of the total CA concentration, and the pH-rate profile displays a maximum at pH 8 and 25°C, which is the pKa of CA. The half-life is 20 min for a CA concentration of 10^{-3} M. The dehydration of the intermediate ketol was shown to be first order with a half-life of 6 hours at pH 8 and 25°C.

In this work, we have measured the rate of the reverse reaction as well as the equilibrium constant. The equilibrium of the CA condensation was

P1.10 continued

EQUILIBRIUM AND KINETICS OF THE ALDOL CONDENSATION

measured spectrophotometrically in H_2O and also in D_2O by ^{1}H-NMR spectroscopy as a function of pH, giving very consistent results. The rate of the retroaldol cleavage, k_{-1}, has been calculated from the measured equilibrium constant, K_{CY}. In the second step, dehydration of Y yields the enone D. The forward and reverse rate constants, k_2 and k_{-2}, as well as the equilibrium constant (K_{YD}) have also been directly measured by UV spectroscopy as a function of pH. The overall equilibrium (K_{CD}) was calculated from K_{CY} and K_{YD}. The equilibrium constant is maximum at pH 7.9 and 25°C ($K_{CD} = 10^8$). The reaction is less favoured as the pH increases. The equilibrium constant falls off below pH 7.9 and is independent of pH below pH 2.



Cyanoacetaldehyde

CA **Y** **D**

These results show that CA dimerization is a very favourable reaction but this does not seem a problem for prebiotic syntheses since pyrimidines are still produced. This suggests that the CA dimer may be an intermediate in these syntheses. The reaction of CA dimer with urea and guanidine needs to be investigated to evaluate this possibility. The role of CA dimer as a useful source of prebiotic compounds other than pyrimidines should also be investigated. The above considerations may be important in aspartic acid synthesis from CA (Miller, 1957).

Ferris, J.P., Zamek, O.S., Altbuch, A.M., Freiman, H. (1974) J. Mol. Evol., **3**, 301-309.

Miller, S.L. (1957) Biochim. Biophys. Acta, **23**, 480-489.

Raulin, F. and Toupance, G. (1975) Bull. Soc. Chim. France, 188-195.

Robertson, M. P. And Miller, S. L. (1995) Nature, **29**, 772-774.

Robertson, M. P., Levy, M., Miller, S. L. (1996) *J. Mol. Evol.* **43**, 543-550.

P1.11

HETEROGENEOUS RADIOLYSIS OF SUCCINIC ACID IN THE CONTEXT WITH CHEMICAL EVOLUTION STUDIES.

M. Colin, S. Ramos-Bernal and A. Negron-Mendoza
Instituto de Ciencias Nucleares, UNAM.
Circuito Exterior, C.U. A.Postal 70-543
México, D.F 04510. México.

Simple carboxylic acids can be considered as key compounds in chemical evolution studies, due to their facile formation under several conditions. Also they were probably important intermediates in the synthesis of complex bio-organic molecules.

For the synthesis of complex molecules a supply of energy was needed. Several sources of energy were effective in generating organic compounds. The comparative importance of several sources on the primordial environment was assessed by Miller and Urey (1959) and their values have been taken since then. There is a common agreement that ionizing radiation energy were minor partners, and they have still a modest place in the approach for prebiotic synthesis. Still, there are many reasons to believe that radioactivity was more abundant that it was thought (Draganic et al.,1990).

As a part of our interest to study the effect of solid state chemistry connected with the catalytic activity of clay minerals, we were prompted to do a systematic investigation on the gamma radiolysis of an heterogeneous system composed by a carboxylic acid (succinic acid)-a clay mineral (Na-montmorillonite)-and water.

For these experiments, 0.1 mol dm-3 aqueous solutions, at their natural pHs, were used. Half of the samples were prepared with 0.5 g of Na-montmorillonite. All the samples were oxygen-free. They were irradiated from 4 to 24 Mrads in a ^{60}Co-source. The quantitative determination of the products was mainly carried out by gas chromatography and gas-chroma-tography-mass spectrometry.

The results obtained shows that from the radiolysis of the system a variety of carboxylic acid were formed from the starting material. Among the acids detected in were malonic, oxalic, succinic, carboxysuccinic, tricarballylic and succinic dimer. Their concentration increased with the

P1.11 continued

dose. The striking difference in both systems was in the amount of products formed In the previous study (Negrón-Mendoza and Ponnamperuma, 1982), it was suggested that the active species in the radiation-induced reactions of the succinic acid resulted from the water radiolytic products. These species, by an indirect attack react with the solute molecules giving rise the observable products. In the presence of clay, the energy is mainly deposited in the clay and then transferred to the solute. This process cause that the decomposition in the acids is in minor extent than without clay.

Our aims has been to stress the relevance of solid surfaces and ionizing radiation, as tools for the study of compounds of potential interest in chemical evolution. In this way, the results obtained underscore this aspect and act as a contributing factor toward the prebiotic synthesis of organic compounds.

This work was partially supported by DGAPA-UNAM and CONACYT grants.

Draganic, I.G., Z.D. Draganic, and J.P. Adloff, 1990: *Radiation and Radioactivity on the Earth and Beyond.* CRC Press, Inc. Boca Raton, Florida.

Negrón-Mendoza, A. and Ponnamperuma, C. 1982, *Origin of Life and Evol. Bios.* **12**, 427.

Miller, S.L. and H.C. Urey, 1959, *Science* **130**, 245.

P1.12

STUDIES OF ADSORBED BIO-ORGANIC SUBSTANCES IN Na-MONTMORILLONITE. A PREBIOTIC VIEW.

A. Guzmán, A. Negrón-Mendoza, Isabel Gamboa de Buen and S.Ramos-Bernal
Instituto de Ciencias Nucleares, UNAM
México, D.F. 04510 México.

Chemical reaction may took place at hydrosphere/lithosphere, atmosphere/ lithosphere interfaces, thus, a reliable simulation of relevant prebiotic environment would require the consideration of multiphase systems. In this sense, minerals that are capable of a selective concentrating and internally binding compounds are of particular interest for evolving molecules. Without such concentration, most prebiotic scenarios come to halt, due that highly concentrate prebiotic environment may be implausible (Sillén, 1965) In particular, some significant fraction of monomers would have formed on mineral surface, or would have been adsorbed there subsequent to prior synthesis. It is very important to elucidate, how much the solid is involve in the chemical transformation that is taking place at its surface. In order to have some idea of the former, some of the properties of the solid had been monitored and correlated with the adsorption of organic compounds.

From the data published in the literature, the role of clays in chemical evolution as selective adsorbed, concentrator and catalyst for polymerization have been demonstreated A review of data on adsorption shows that for most compounds with biological relevance; the adsorption is larger in acidic pH. Sometimes the binding of these types of compounds drops to almost null adsorption at pH 8, since the pH of the primitive ocean is believed was pH 8 this may disqualify some results. Nevertheless, there are several natural conditions that attain higher acidities, and thus improve adsorption binding, supporting in this way the possible role of microenviroments in the primitive Earth.

In this work we present experimental results from ultraviolet spectroscopy, thermoluminescence, FTIR spectroscopy and X-rays obtained from samples with adenine and Poly A adsorbed in Na-montmorillonite, submitted to various treatments that included the selective blocking of the

P1.12 continued

interlamellar channel with different compounds like hexadeciltrimethyl ammonium salt and with several glycols changing the carbon chain from C_2 to C_{10}. The results obtained indicate that both compounds are readily adsorbed into the clay.

Adenine is adsorbed in the interlamellar space via ionic interchange and Poly A is bonding mainly at the edges of the crystal. The thermoluminiscent signals allow us to support the proposal that defects in solids caused by natural radiation may serve as energy transfer for reactions in the clay.

This work was partially supported by CONACYT grant.

Sillén, L.G. 1965, *Arkiv for Kemi* **24**, 431-456.

P1.13

THERMAL PREBIOTIC OLIGOMERIZATION OF AMINO ACIDS: A MARKOV CHAIN APPROACH.

F.G. Mosqueira[1] , S. Ramos-Bernal[2], A. Negrón-Mendoza[2] and R.M.A. Estrada-Ramírez [2]
Dirección General de Divulgación de la Ciencia, UNAM, 04510 México D.F. México.
[2]Instituto de Ciencias Nucleraes, UNAM, 04510 México D.F. , México

In this work, we consider a simple mathematical model to simulate a prebiotic oligomerization process. From the physicochemical characteristics of the constitutive monomers, using mainly electrostatic considerations, we analyze the synthesis of trimers and small oligopeptides.

The mechanism of oligomerization of a particular trimer is known (Hartmann et al., 1981). The first kinetic step is the cyclization of glutamic acid yielding pyroglutamic acid . In a second slower stage the other amino acids form a cyclic diketopiperazine that further reacts with the pyroglutamic acid to produce a trimer.

We consider the thermal polymerization process as a discrete set of states in which the reactive end of a growing polymer consisting of n-bonded monomers increases to (n+2) bonded monomers. In the initiation state, n= 1. The main assumption is that the transition probability of incorporating the amino acid into the polymer is influenced only by the interaction between the incoming aminoacid dimer and the n-aminoacid in the reactive end of the polymer, and is not influenced by any other previous monomers n-1, n-2, ... already bonded in the n-polymer. Such physicochemical assumption goes nicely with the definition of a Markov Chain, that we use to model the thermal oligomerization of amino acids:

$$\text{Prob}\,(X_{T+1} = m | X_0 = a, X_1 = b, ..., X_T = k) = \text{Prob}(X_{T+1} = m \,|X_T = k) \qquad (1)$$

where a,b, ..., m are arbitrary designations of the states assumed by system X at time 0, 1, ..., T, T+1, ...
Under such construction, it is possible to follow the dimer additions to the polymer and calculate the possible sequences allowed. To this end we use the following equation:

P1.13 continued

THERMAL OLIGOMERIZATION OF AMINOACIDS: A MARKOV CHAIN APPROACH.

$$P(n) = AP(n-1) \qquad (2)$$

where P(n) and P(n-1) are the vectors of the n and n-1 states, respectively, and A is the transition or reactivity matrix with elements a_{ij}.
We assume also that the reactivity between amino acids will depend entirely on their electrical properties, and probably on volume . We clasify aminoacids into four groups: polar positive, polar negative, neutral, and non polar; in accordance with previous definitions (Dickerson and Geis, 1969). Their pairwise interaction ij between electrical different aminoacids i and j are given numerical values, a_{ij}. We assume also that the transition matrix is symmetric, i.e., the value of the matrix element for interaction a_{ij} is the same as for a_{ji}.
The iterative application of equation (2) will generate the theoretical sequences that we expect to model those sequences produced experimentally.
This work was partially supported by CONACYT grant.

Dickerson, R.E. and Geis, I., 1969. *The Structure and Action of Proteins.* Harper & Row Publishers, New York.
Hartmann, J., Brand, M.C., and Dose, K. ,1981. *BioSystems* **13**, 141-147.

P1.14

TD-NMR TITRATION STUDIES ON COMPLEXATION OF BORATE WITH POLYHYDROXYLATED ORGANIC COMPOUNDS. IMPLICATIONS FOR CHEMICAL EVOLUTION AT HIGH TEMPERATURES

Vily Marius Cimpoiasu, Gyury Steinbrecher, Romulus Scorei, Iulian Petrisor, Ion Brad, Ion Olteanu, Liana Simona Sbirna

University of Craiova, A.I.Cuza 13,1100 Craiova, ROMANIA

The presence of polyhydroxylated compounds in the prebiotic environment may be an important source for the "RNA World". Although this hypothesis has been accepted, the problem of thermal stability of the polyhydroxylated compounds (polyols) on the early Earth is still opened.

Our research refers to the borate specificity in the formation of the cis-diol complex with a high equilibrium constant. The time-domain ^{1}H-NMR titration methods are used to monitor the solute concentration by the linear dependence of the "spin-spin" relaxation rate. This technique also permits to establish the specific "spin-spin" relaxation times for the water-anomers compartments, because the difference in the solvation energy induces the different molecular motion (reflected in the "spin-spin" relaxation times of water protons in a fast exchange process). The comparative analysis was performed on the polyols and borate-polyols systems in the thermal decomposition process. In the presence of borate anions, the anomers form cis-diol complexes.

Our measurements show that the half-life decomposition time is in accordance with other researchers' measurements and is equal to half-life time for the alpha and beta anomers in a normal glucose solution. By monitoring the B-complexes during the thermal decomposition process (85^0C) the experimental results show the existence of an interaction between the polyols decomposition products and borate anions through the formation of certain complexes which obstruct the open-chain form of polyols. These complexes also decrease the decomposition rate to zero.

Therefore, in presence of boron, the ribose and other sugars could be the components of the first genetic material under prebiotic conditions at high temperature.

P1.15

PREBIOTIC AND PROTOBIOTIC MOLECULAR MACHINE PROCESSES IN INTERSTELLAR MEDIUM

G. Cocho* and G. Martinez-Mekler+
* Instituto de Fisica, UNAM, Apdo. Postal 20-364, 01000 Mexico DF Mexico
+ Centro de Ciencias Fisicas, UNAApdo. Postal 48-3, Cuernavaca, Morelos, Mexico

The presence of a variety of organic compounds in the interstellar medium, comets and meteorites (Kissel and Kruger (1987), Greenberg (1995)), as well the recent evidence for life on Earth 3870 million years ago (Mojzis et al. (1996)), suggests a scenario where prebiotic and perhaps protobiotic processes took place outside of the Earth. It has been proposed that polymerization processes could take place in the interstellar medium, with ultraviolet ionizing radiation being the source of monomer activation and long wave electromagnetic radiation forcing the activated monomers to oscillate, collide and form polymers from free radical covalent bonding (Martinez-Mekler et al. (1996), Aldana et al. (1998)). If the polymers have interaction in channels through asymmetric potential, there will be a systematic motion, cleaning the channels, allowing additional polymerizations, and it has been shown that under rather general conditions the polymer slow down every three monomers, being perhaps an important dynamical element in the origin of the three bases for codon in the genetic code. The locomotion would be due to molecular machines working in quasi-unidimensional channels, tubes or filaments, what is consistent with the structure of interstellar medium (Greenberg (1995)). As a matter of fact, important biological processes, as replication, transcription, translation, and cytoskeleton dynamics are quasi-unidimensional. Low temperature minimum energy considerations favor the formation of extensive ordered oriented sequence segments in order to reduce cost energetic boundaries between such segments, and the preferential chiral destruction of domains of one type, by polarized ultraviolet radiation might be the origin of biological chirality. One could envisage situations where two interacting polymer strands are more or less complementary and move along a channel that eventually bifurcates. The strands could separate and branch, with possibility of reconstituting the complementary strand, hence implementing a rudimentary replication procedure. In the transit to Earth, an open problem is the switching from electromagnetic to chemical driving force. At present ATP energy is transformed in protonic electric gradients that might be related to these primeval interstellar molecular machines.

REFERENCES.

Aldana, M., Cazarez-Bush, F., Cocho, G. and Martinez-Mekler, G. :1998, Physica A 257, 119.
Greenberg, J.M. : 1995. AIP Proc. 379, 185.
Kissel, J. and Kruger, R. :1987, Nature 326, 755.
Martinez-Mekler, G., Aldana, M., Cazarez-Bush, F., Garcia Pelayo, R. and Cocho, G. :1996, Origins of Life and Evolution of the Biosphere. (In press).
Mojzis S.J. et al. :1996, Nature 384,55.

P1.16

ROCKS AS PROGENITORS OF LIFE? ORGANIC SYNTHESIS IN THE SOLID STATE

Friedemann Freund, NASA ARC 239-4 Moffett Field, CA 94035

Most chemical synthesis pathways studied to date involve reactions in gas and liquid phases, at gas- or liquid-solid interfaces or by "soft" intercalation into clays. By contrast, the "hard" matrix of igneous minerals has not been considered a viable, potentially powerful reaction medium for the synthesis of complex organic molecules.

When minerals crystallize from fluid–laden magmas or recrystallize in a fluid–laden metamorphic environment, they invariably dissolve small amounts of the H_2O, CO_2 and N_2 components which make up the fluid phase. Upon entering the "hard" mineral matrix these fluid molecules do not retain their molecular identity. Instead, they "melt" into the mineral matrix in form of oxyanion complexes. Little is known about the nature of such oxyanions at near-magmatic temperatures but, upon cooling, a fascinating reaction takes place: a reshuffling of electrons among the lattice oxygen anions on one side and the low-z elements H, C and N on the other side. This causes oxygen to become oxidized to the O^- state and the low-z elements to become reduced to H, $C^{\delta+}$ and $N^{\delta+}$. Importantly, this happens below 500°C when the minerals have already drifted out of thermodynamic equilibrium. It allows oxidized O^- to coexist with reduced low-z elements and reduced metal cations. The next step is also thermodynamically prescribed: The segregation of the reduced low-z species into dislocations and other major defects, driven by the strain differential. The segregation acts like a big broom sweeping the low-z elements into the various defect sites where they begin forming C–C, C–H, C–O and C–N bonds under the 3–dimensional constraints of the local lattice environment. As long as these polyatomic entities are encased in their host mineral matrices, they cannot yet be called "molecules". However, when liberated from their tight embrace, for instance during weathering, they turn into organic molecules.

Compounds solvent–extracted from synthetic MgO and upper mantle olivine crystals comprise carboxylic and dicarboxylic acids (oxalic, malonic, glycolic, succinic), fatty acids (C_6 to C_{12}), urea and glycolamide. The yields per unit volume rock, though still uncertain, may be as high as 10–100 ppm C_{org} per lattice O^{2-}. Assuming weathering rates of 1–10 km^3/year on the early Earth, this would translate into C_{org} production rates of the order of 10^{10}–10^{12} g/year, possibly augmented through leaching of rocks by hydrothermal fluids. Since such a process would continue and even increase over time, it represents a substantial source of complex compounds, contributing to the pool of organics from which Life arose. The process would also occur on any other rocky planet with liquid water.

P1.17

WAS THE RADIOACTIVITY THE MOST IMPORTANT ENERGY SOURCE IN THE PREBIOTIC SYNTHESIS?

Leon Garzon, Departamento de Energia, Independencia,13, 33004 Oviedo;
e- mail:lgarzon.relay.etsimo.uniovi.es

Coming from the solar nebula, in the formation of the Earth, the radioactive nuclides, ^{238}U, ^{235}U, ^{232}Th and the ^{40}K concentrated preferably on the mantle and terrestrial crust, undergoing a considerable increase in their concentrations. The first three nuclides originated three radioactive families. They are widespread in the terrestrial crust down to the reached depths (some Kms). In the past the abundances were superior to the present ones, according to the radioactive decay.Such nuclides were obviously in the grains, within which, therefore, a continuous production of particles, photons and daughters took place. The most important daughters are the radioactive inert gases (radon isotopes); not all the atoms of the radon isotopes produced in the disintegrations could emigrate towards the exterior of the grains, but a certain fraction η (emanating power), which could incorporate into the volume of the voids,where it would continue dissipating its energy. Simultaneously, the remaining fraction,1-η, would do it inside the grains. The values of η are practically null for ^{219}Rn and ^{220}Rn because they would disintegrate totally before being liberated from the lattices. So, two types of sources exist in the crust:

The source in the grains, S_g, and the source in the voids, S_p. For the continental crust the inventories for h=1 km (about 4 Ga ago) are $Sg=3\times10^{19}\ Ja^{-1}$ and $Sp=3\times10^{18}\ Ja^{-1}$, which are comparable with those of the electric discharges (Miller et.al.,1976; Chyba and Sagan,1992).

On account of the exchanges between the crust and the atmosphere, the components of the latter were continually irradiated. The presence of water, certain inorganic catalysts, Fe^{2+} cations, along with the protection against the ultraviolet solar radiation, the ubiquity and the availability of the sources, allow us to suppose that the radioactive sources were the most relevant of all in directing the prebiotic synthesis. In the uranium deposits of the same age, the prebiotic synthesis would be carried out with even more facility.

Chyba, C.F. and Sagan, C.: 1992, Nature **355**,125.
Miller, S.L., Urey, H.C. and Oro, J.: J. Mol. Evol. **9**,59.

P1.18

EVIDENCE THAT LIFE ORIGINATED IN A SULFUR ENVIRONMENT

Daniel L. Gilbert
Unit on Reactive Oxygen Species, BNP, NINDS, NIH, Bethesda, MD 20892-4156

The major elements in the universe are hydrogen, helium, oxygen, carbon, and nitrogen. These elements, with the exception of the chemically inert helium, are also the most abundant ones in the biosphere (Gilbert, 1960, 1996). Although sulfur is not so abundant, it is important as a redox regulator. Stressful agents, such as reactive oxygen species (ROS), activate transcription factors, such as NF-kappa B, AP-1, Fnr, SoxR, and OxyR, which in turn activate antioxidant genes. These factors all contain sulfur and possess the ability to undergo redox reactions. When ROS is encountered, the transcription factor is activated, which then activates antioxidant genes. The genes then promote a specific type of antioxidant defense; i.e., superoxide dismutase, catalase, etc. These antioxidants decrease the ROS concentration and no longer act as a signal for the transcription factor, which in turn no longer activates the antioxidant genes resulting in an increase of the ROS concentration. Thus, oscillatory regulation of ROS is achieved. Burton, Williams, and Norris (1995) experimented on *Sulfolobus*, a type of archaebacteria which uses sulfur as its energy source. When ferrous iron was used as the energy source, a protein was produced that showed significant similarity to thiol antioxidant proteins. Woese (1987) has postulated that life began in a sulfur rich high temperature environment. *Sulfolobus* is an organism which requires high temperature and low pH. Therefore, we postulate that many of the transcription factors could have evolved from organisms similar to *Sulfolobus*, and that life originated in a sulfur environment.

Burton, N.P., Williams, T.D., and Norris, P.R.: 1995, FEMS Microbiol. Lett. **134**, 91.

Gilbert, D.L.: 1960, Perspect. Biol. Med. **4**, 58.

Gilbert, D.L.: 1996, in M.J. Fregly, and C.M. Blatteis (ed.), *Handbook of Physiology, Section 4. Adaptation to the Environment. Vol. II*, Oxford University Press, New York, 1059.

Woese, C.R.: 1987, Microbiol. Rev. **51**, 221.

P1.19

NITROGEN OXIDES AS POSSIBLE ACTIVATING AGENTS FOR PREBIOTIC PHOSPHORYLATION.

William J. Hagan, Jr., Timothy R. McAuley and Lucio R. Volino
School of Mathematics and Sciences, College of St. Rose, 432 Western Ave., Albany, NY 12203-1490 USA; haganw@rosnet.strose.edu.

Dinitrogen trioxide (N_2O_3) has been studied extensively as a nitrosating agent of organic substrates, including alcohols, thiols and amines (Williams, 1988). Two isomeric forms of N_2O_3 are known, one from the combination of NO with NO_2 (producing $ONNO_2$) and the other (ONONO) from acidification of aqueous nitrite solutions. Significantly, inorganic phosphate at pH 7 competes efficiently with other nucleophiles in a reaction with $ONNO_2$ that leads to partial loss of O-18 from isotopically labeled orthophosphate, consistent with the formation of nitrosophosphate ($ONOPO_3^{2-}$) as an intermediate (Lewis *et al.*, 1995; DeMaster *et al.*, 1997):

$$HOPO_3^{2-} + ONNO_2 \rightarrow ONOPO_3^{2-} + NO_2^- + H^+$$

The polarizability of nitrite, along with a weak basicity ($pK_a^{25} = 3.3$), would make it a good leaving group, an expectation supported by the observation of hydrolysis at the phosphorus position in nitrosophosphate (DeMaster *et al.*, 1997). In addition, we have recently carried out semi-empirical (AM1) molecular orbital calculations on the isomeric forms of dinitrogen trioxide to show that $ONNO_2$ lies 26.7 kcal/mol in energy *above* ONONO, indicating that the asymmetric structure is a significantly more activated species. Experimental data will be presented on the ability of nitrosophosphate to promote the formation of phosphate esters under plausible prebiotic conditions, and possible sources of N_2O_3 on the early Earth will be addressed.

DeMaster, E. G., Quast, B. J., and Mitchell, R. A.: 1997, Biochem. Pharm. **53**, 581-5.
Lewis, R. S., Tannenbaum, S. R., and Deen, W. M.: 1995, J. Amer. Chem. Soc. **117**, 3933-9.
Williams, D. L. H.: 1988, *Nitrosation*, Cambridge University Press, p. 24.

P1.20

THE ORIGIN AND EVOLUTION OF PROTEIN SYNTHESIS

Hyman Hartman
IASB, 28 Banks St, Cambridge, MA 02138

"Life has developed its processes gradually, never rejecting what it has built, but building over what has already taken place. As a result the cell resembles the site of an archeological excavation with the successive strata on top of one another, the oldest one the deepest" (Szent-Gyorgyi, 1972). It is therefore in the prokaryotic and eukaryotic cell that the excavation of the origin and evolution of protein synthesis must take place.

The molecular archeologist must now begin to excavate the cell for the evolutionary history of the ribosome and RNA-coded synthesis of proteins.

What cell has the key to the history of the ribosome? It is in the eukaryotic cell that the remnants of an RNA-based cell which did not have a ribosome are to be found (Hartman, 1992). What preceded the ribosome? It was preceded by the first membrane, which was a protein RNA complex. At some point in cellular evolution, the primitive membrane must have evolved into the ribosome and the initiating, elongating, and terminating factors. The remnants of this membrane are to be found in the cytoskeleton of the eukaryotic cell and the small RNAs which are found in great numbers in the eukaryotic cell (Hartman, 1992).

The evolutionary record of the genetic code is partially contained in the structure and sequences of the aminoacyl-tRNA synthetases (Hartman, 1995a). Recent work on the synthetases support the hypothesis "that the present-day aminoacyl-tRNA synthetases have originated from ancestral forms that were involved in noncoded thioester-dependent peptide synthesis, functionally similar to the present-day nonribosomal peptide synthesis by multi-enzyme thiotemplate systems" (Jakubowski, 1998). This would imply that the first synthesis of peptides in the primitive living system was not coded for by RNA.

The polynucleotide directed polymerization of amino acids into polypeptides by means of a genetic code is a later development. These early evolutionary events took place in an evolving system of replicating and mutating iron-rich clays (Hartman, 1998).

P1.20 continued

THE ORIGIN AND EVOLUTION OF PROTEIN SYNTHESIS

Hartman, H.: 1992 The Eukaryotic Cell and the RNA-Protein World in *Frontiers of Life*
Eds J and K Tran Thanh, J.Mounolou, J. Schneider, and C. Mckay.
Published by Editions Frontieres p. 163
Hartman, H.: 1995a *Origins Life. Evol. Biosphere* **14**, 643.
Hartman, H.: 1995b *J. Molec. Evolution* **40**, 541.
Hartman, H.: 1998 *Origins Life. Evol. Biosphere* **28** , 515.
Jakubowski, H.: 1998 *Biochemistry* **37,** 5147.
Szent-Gyorgyi, A.:1972 The Living State, Academic Press: New York P.6.

P1.22

MODIFIED PURINES FROM THE POLYMERIZATION OF HYDROGEN CYANIDE

Matthew Levy* and Stanley L. Miller
Dept of Chemistry/Biochemistry, University of California, San Diego
La Jolla, CA 92093-0506, USA

*present address: Inst. for Cellular & Molecular Biology, The University of Texas at Austin, Austin, TX 78712, USA

The prebiotic synthesis of purines from hydrogen cyanide (Oro, J., 1960; Oro J. and Kimball A. P. 1961) is well established together with a number of modified pathways (Ferris J. P. and Orgel L. E., 1966; Sanchez R. A. *et al.* 1967). In view of the possible importance of modified purines in the origin of the first replicating systems, we searched an ammonium cyanide polymerization for presence of m^6A (6-N-methyl adenine), a modified purine that is found in almost all forms of RNA as well as DNA (Limbach, P. A. *et al.* 1994). No m^6A could be detected (<0.0005% compared with 0.02% adenine). Because it seems unlikely that cyanide polymerization reactions taking place on the primitive Earth would contain solely ammonium cyanide, we tried the polymerization in the presence of CH_3NH_2, a prebiotic amine that is found in the Murchison meteorite (Jungclaus G. *et al.* 1976). A mixture of ammonium (7.5M) and methylammonium (2.5M) cyanide was heated to 70 °C for 18hr. Upon acid hydrolysis this gave a 0.02% yield of A, a 0.01% yield of m^6A, and 0.009% m^9A.

The production of m^9A from the reaction of HCN and CH_3NH_2 suggested that the polymerization hydrogen cyanide in the presence of glycine might produce the modified purine adenine 9-acetic acid, a component of the possible alternative genetic material PNA (Nielsen P. E., 1993 and Miller S. L., 1997). A solution of 7.5M NH_4CN and 2.5M glycine was heated to 80 °C for 18hrs. Upon hydrolysis this gave 0.0051% A, 0.0013% N-(6-purinyl)glycine and 0.0062% adenine 9-acetic acid.

Because cyanide polymerization reactions would be limited to cold areas of the Earth, where cyanide could be concentrated through eutectic freezing, we also analyzed a solution containing 0.075M ammonium cyanide, and 0.025M methylamonium cyanide which had been frozen at -20 °C for 3 months. This gave a 0.001% yield of A, and a 7.4×10^{-4}% yield of m^9A (no m^6A was detected $< 1 \times 10^{-5}$%).

These results demonstrate that a wide variety of modified purines can be generated from the prebiotic cyanide polymerization reaction. Many of these purines occur widely in the tRNA and rRNA of all organisms suggesting that they may have played an important role in the RNA world. In addition the synthesis of adenine 9-acetic acid suggests that this base would have been available for use in the pre- RNA world.

P1.22 continued

MODIFIED PURINES ... Levy and Miller, Contd.

Ferris J.P., Orgel L.E. :1966, J. Am. Chem. Soc. **88**, 1074.
Jungclaus G., Cronin J.R., Moore C.B., Yuen G.U. :1976, Nature **261**,126.
Limbach P.A., Crain, P.F., McCloskey J.A. :1994,Nucleic Acids Res.**22**, 2183.
Miller S.L. :1997, Nature Struct. Biol. **4**, 167.
Nielsen P.E. :1993, Origins Life Evol. Biosphere **23**, 323.
Oro, J. :1960, Biochem. Biophys. Res. Comm. **2**, 407.
Oro J., Kimball A. P. :1961, Arch. Biochem. Biophys. **94**, 217.
Sanchez R. A., Ferris J. P., Orgel L. E. :1967, J. Mol. Biol. **30**, 223.

P1.23

A NOVEL PREBIOTIC SYNTHESIS OF AMINO ACIDS

Quinn Maughan and Stanley L. Miller
Department of Chemistry and Biochemistry
University of California, San Diego
La Jolla, California 92093-0506, USA

The origin of the metabolic pathways is usually considered to be by variations of the Horowitz hypothesis (Horowitz 1945) in which the pathways are built up a step at a time by transforming compounds from the prebiotic soup. Thus it is likely that many of the steps in biosynthetic pathways were initially non-enzymatic reactions. Known prebiotic synthesis of amino acids largely proceed by a Strecker synthesis although there may be exceptions. On the other hand, biosynthetic pathways use transamination or reductive amination by NADH or NADPH. Since NADH is a complex coenzyme and needs an enzyme for activity, the question becomes what was the bridge between these two pathways? One possibility is the reaction of keto-acids + ammonia to give amino acids. This is an old reaction (Erlenmeyer & Kunlin 1899; De Jong 1900; Erlenmeyer & Kreutz 1904) and has been studied in a prebiotic context by Yanagawa (Yanagawa *et al.*, 1982; Yanagawa *et al.*, 1984).

An example of the reaction is

$$2 \text{ pyruvate} + NH_3 \text{ ---> alanine} + \text{acetate}$$

where one pyruvate is reduced to alanine at the expense of the second being oxidatively decarboxylated to acetate. Some of the acetate is incorporated in an amide linkage on the alanine.

We have found that this reaction produces amino acids in yields as high as 40%. The reaction is first order in ammonia, second order in α-keto acid, and has a complex pH rate profile. This may have been a productive prebiotic synthesis of amino acids provided there was an adequate source of α-keto acids.

De Jong, M.: 1900, *Rec. Trav. Chim.*, **19**, 259-310.
Erlenmeyer, E. & Kreutz, A.: 1904, *Leibig Ann.*, **337**, 205-221.
Erlenmeyer, E. & Kunlin, J.: 1899, *Leibig Ann.*, **307**, 146-162.
Horowitz, N.H.: 1945, *Proc. Natl. Acad. Sci.*, USA, **31**, 153 - 157.
Yanagawa, H., Makino, Y., Sato, K., Nishizawa, M., & Egami, F: 1984, *Origins Life*, **14**, 163 - 169.
Yanagawa, H., Makino, Y., Sato, K., Nishizawa, M., & Egami, F: 1982, *J. Biochem. (Tokyo)*, **91**, 2087-2090.

P1.24

DESIGN OF THE COSMOBIOLOGY EXPERIMENTS IN EARTH ORBIT TO TEST ABIOTIC FORMATION OF BIOORGANIC COMPOUNDS

Hirofumi Hashimoto[1], Kentaro Ushio[2], Takeo Kaneko[2], Kensei Kobayashi[2], Andre Brack[3], Luigi Colangeli[4], J. Mayo Greenberg[5], Gerda Horneck[6], Akira Kouchi[7], Rafael Navarro-Gonzalez[8], Francois Raulin[9], Takeshi Saito[10], and Masamichi Yamashita[11]

[1]Institute of Engineering Mechanics, University of Tsukuba, Tsukuba, Ibaraki 305-8573, Japan; [2]Yokohama National Univ., Japan; [3]CNRS, France; [4]Astron. Obs. of Capodimonte, Italy; [5]Leiden Univ., The Netherlands; [6]DLR, Germany; [7]Hokkaido Univ., Japan; [8]Mexico Nat. Autonomy Univ., Mexico; [9]Paris Univ., France; [10]Tokyo Univ., Japan; [11]ISAS, Japan.

Organic compounds delivered by comets to our planet are of interest, since they might have been a source of organic materials for the terrestrial biosphere. Those compounds have an interstellar origin. Extensive studies have been conducted on the ground to simulate the formation of bioorganic compounds under the interstellar medium conditions. Bioorganic compounds such as amino acids were detected in the products by irradiation of ice mixtures of CO (or CH_4, CH_3OH), NH_3 and H_2O with high energy particles or UV light. As a logical extension of the study, those findings should be confirmed in real space conditions. A conceptual design was developed for a cosmobiology experiment. The experimental system consists of a cryogenic system to keep solidified gas sample, and an optical device to select and amplify the ultraviolet part of the solar light for irradiation. The removal of light at longer wavelength, which is ineffective to induce photochemical reactions, reduces the heat load to the cryogenic system that holds solidified reactants including CO as a constituent species of interstellar materials. Other major hardware components were also defined in order to achieve the scientific objectives of this experiment. Those are a cold trap maintained at liquid nitrogen temperature to prevent the contamination of the sample during the exposure, a mechanism to exchange multiple samples, and a system to perform bake-out of the sample exposure chamber. This experiment system is proposed as a candidate payload implemented on the exposed facility of Japanese Experiment Module on International Space Station.

P1.25

ABIOTIC SYNTHESIS OF AMINO ACIDS FROM SIMULATED PRIMITIVE EARTH ATMOSPHERE WITH HIGH ENERGY PARTICLES AND SYNCHROTRON RADIATION

Kensei Kobayashi, Hitomi Masuda, Takeo Kaneko, Jun-ichi Takahashi*, Teruo Hosokawa* and Takeshi Saito**
Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan
* NTT Telecommunications Energy Laboratories, Atsugi 243-0198, Japan
** ICRR, University of Tokyo, Tanashi 188-8502, Japan

It is strongly suggested that the primitive atmosphere of the earth was composed of CO_2, CO, N_2 and H_2O. It is not easy to form amino acids from this kind of slightly reduced gases by electric discharges or near ultraviolet lights. We performed simulation experiments to examine the roles of cosmic ray and short-wavelength solar radiation in abiotic formation of amino acids.

A gas mixture of CO_2, CO and N_2 at various mixing ratios, simulating the primitive earth atmosphere, was enclosed in a glass tube with liquid water. The gas mixture was irradiated with protons of 3.0-4.0 MeV generated from a van de Graaff accelerator (Tokyo Institute of Technology), protons of 40 MeV and He^{2+} of 65 MeV from an SF cyclotron (Institute for Nuclear Study (INS), University of Tokyo), electrons of 0.4-1 GeV from an electron synchrotron (INS, University of Tokyo), γ-ray from a ^{60}Co source (University of Tokyo), and soft X-ray or vacuum UV light in synchrotron radiation (NTT). The aqueous products were acid-hydrolyzed and then analyzed by HPLC and/or GC/MS.

When the gas mixtures containing CO and N_2 was irradiated with any of high energy particles, amino acids were obtained in high yield when the products was hydrolyzed: The G-Value of glycine when an equimolar mixture of CO and N_2 was ca. 0.02. Gamma ray gave almost the same G-value as particles, and X-ray (ca. 1.5 keV) gave less G-value (0.004) than them. When vacuum UV light was applied to the gas mixture, amino acids detected was only as much as background level.

The present results, with estimation of energy flux in primitive Earth, suggest that cosmic ray was the most effective energy source for the formation of amino acid precursors in the primitive earth atmosphere, followed by soft X-ray region of solar radiation.

P1.26

SYNTHESIS OF BIOORGANIC MOLECULES FROM A CO-N_2-H_2O GAS MIXTURE USING A MAGNETO-PLASMA DYNAMIC ARC-JET

Shin Miyakawa[1], Ken-ich Murasawa[2], Kensei Kobayashi[2]
and Akira B. Sawaoka[1]
[1]Materials and Structures Laboratory, Tokyo Institute of Technology, 4259, Nagatsuta, Midori, Yokohama 226-8503, Japan
[2]Department of Chemistry and Biotechnology, Faculty of Engineering, Yokohama National University, Hodogaya, Yokohama 240-8501, Japan

It is very likely that the early Earth's atmosphere was mainly composed of CO_2, N_2, H_2O on average (Kasting, 1993). Little bioorganic compounds are, however, synthesized from the spark discharge in a CO_2-N_2-H_2O gas mixture. This is a severe problem in the field of chemical evolution.

When comets and carbonaceous asteroids collided against the early Earth, volatile compounds or organic compounds in these impactors were vaporized and recombined to form a new atmosphere in which carbon monoxide is likely to have exceeded carbon dioxide (Kasting, 1990). Bolide impacts might locally and temporarily make a favorable circumstance for the chemical evolution.

In the present study, a high-temperature plasma was produced from a CO-N_2-H_2O gas mixture using a magneto-plasma dynamic arc-jet (Miyakawa *et al.*, 1997, 1998) in order to simulate the post-impact plume. Bioorganic compounds such as amino acids were detected in the synthesized solid material after acid hydrolysis. These compounds were more efficiently formed than in the spark discharge experiment. Impacts of comets or carbonaceous asteroids may have been important for the origin of life.

Kasting, J. F.: 1990, Origins Life Evol. Biosphere **20**, 199.
Kasting, J. F.: 1993, Science **259**, 920.
Miyakawa, S., Tamura, H., Kobayashi, K. and Sawaoka, A. B.: 1997, Jpn. J. Appl. Phys. **36**, 4481.
Miyakawa, S., Tamura, H., Kobayashi, K. and Sawaoka, A. B.:1998, Appl. Phys. Lett. **72**, 990.

P1.27

MINERAL-WATER REACTIONS AS SOURCES OF MOLECULAR HYDROGEN AND ABIOTIC ORGANIC MATTER

Nils G. Holm
Department of Geology and Geochemistry, Stockholm University,
S-106 91 Stockholm, Sweden

In oceanic lithosphere Fischer-Tropsch Type (FTT) synthesis of organic compounds is probably a significant process. In the commercial Fischer-Tropsch synthesis reaction organic compounds, especially alkanes, alcohols and carboxylic acids, are formed at high temperature from CO and H_2 in the presence of a metal or mineral catalyst. On Earth, the mantle is degassed with respect to CO and CO_2, but these gases may also originate from other sources like, for instance, dissociation of bicarbonate in sea water. During transformation of the rock peridotite to serpentine Fe(II) in its minerals is oxidized partially. Peridotite is an ultramafic rock, i.e. a rock with low silica content, that originates from the Earth's upper mantle. When the Fe(II) minerals are oxidized to magnetite during the serpentinization process molecular hydrogen reduced from water will be an important reaction product. The hydrogen may then react with CO and CO_2 and form organic compounds. Slow-spreading (fractured) ridges like the Mid-Atlantic Ridge (MAR) and the SW Indian Ridge (SWIR) are more likely to reveal abiotic production of organic compounds than fast-spreading ones because of better penetration of water. The classes of organic compounds that are predicted to form in relatively high quantities in these environments are straight-chain hydrocarbons and carboxylic acids.

Fluids of two hydrothermal systems of the Rekjanes Peninsula on Iceland have been sampled. Mixed marine-meteoric hydrothermal waters were collected from the wellhead of one drilled well of the Reykjanes system (290°C) at the tip of the peninsula and two wells of the Svartsengi field (240°C) further to the east. The bedrock consists of basalts and the Fe(II)-minerals are dominated by pyroxenes. Organic compounds potentially present in the fluids were concentrated on different types 'sorbent extraction units'. Analysis results by GC-MS show the presence of C_8-C_{16} monocarboxylic acids at both 240 and 290°C (although the concentrations were much higher at the low temperature). The presence of primarily carboxylic acids is in accordance with thermodynamic calculations, which predict a metastable maximum concentration of carboxylic acids at about 200°C under the redox conditions buffered by the PPM (pyrite-pyrrhotite-magnetite) mineral assemblage. The reason why the samples lack shorter carboxylic acids than octanoic acid is due to

P1.27 continued

the fact that phase separation occurred at the pressure release during sampling and short acids were expelled with the steam phase.

Recently we analyzed fluid samples collected at 364°C in the Rainbow Hydrothermal Field of the Mid-Atlantic Ridge for their organic constituents in the same way as the Icelandic fluids. The Rainbow Hydrothermal Field is special in the sense that it occurs in outcrops of the ultramafic peridotite. The Fe(II) minerals are dominated by olivine. The redox conditions are buffered by the FMQ (fayalite-magnetite-quartz) mineral assemblage. GC analyses by Charlou and colleagues (1998) have revealed high concentrations of H_2 (13 mmol/kg) and CH_4 (2.2 mmol/kg). Our GC-MS analyses of the extracted organic matter showed that the major component of organic compounds consists of straight-chain hydrocarbons with chain lengths of 16-30 carbon atoms.

Charlou, J.L., Fouquet, Y., Bougalt, H., Donval, J.P., Etoubleau, J., Jean-Baptiste, Ph., Dapoigny, A., Appriou, P., and Rona, P.A.: 1998, Geochimica et Cosmochimica Acta **62**, 2323.

P1.29

JUST A MOMENT BEFORE THE ORIGIN OF LIFE

O.P.H. Khandelwal, 101, Happy Apartment, 16 E, Sadhna Nagar,
Indore - 452005 (M.P.), INDIA, e-mail : o_khandelwal@hotmail.com

Key Word : Evolution of matter, origin of life, force, energy, matter and co-ordination.

All the Ingradients which are supposed to constitute a life were accumulated in a pot or vessel but life wasn't manifested in them. We can just imagine the state which existed a moment before the evalution of life in a bialayer vesical (Deamer, 1997) or co-acervates (Oparin, 1924) or mineral surface (Wächtershäuser,1988). The chemicals were 20, aminoacids, **mRNA, tRNA, rRNA, RNApolymerase**, ribosome, minrals like $\mathbf{Fe^{2+,3+}}$, $\mathbf{Mg^{2+}}$, $\mathbf{Mn^{2+}}$ and all five nucleosides, and bases [adenine, guanine, cytosin, uracil and thymine] sugar, phosphate and many other enzymes needed to form life. All these were ready to transcribe and translate the 'Protein Synthesis'. Bio-energetics chemical like **ATP** and **ADP** were also present in this condition. The matter, energy, and force were present. But there was no co-ordination among them to start life. As soon as all the ingradients (biochemicals and bioenergetics) got co-ordinated. The life got evolved for the first time. According to Shri Aurobindo, an Indian philosopher, "So it is throughout evolutionary Nature; **M**atter couldn't have become animate if the principle of **L**ife hadn't been there constituting **M**atter and emerging as a phenomenon of **Life-in-Matter**" (Aurobindo, 1963; Johan, 1991). Therefore some other principle was further needed to co-ordinate among the matter, energy and force for evolution of life.

Let us hope that in future we may be able to confirm this theory by experiments. The perspective concerens the chemistry of the prebiotic Earth : (Deamer, 1997) which organic molecule were plausible avilable. How did they assemble into a complete system that led to the first molecular assemblage was able to reproduce their own structure ? (Chyba, 1990; Deamer, 1997; Miller, 1959; Oro 1992).

All these molecules were not able to enough to evolve life all by themselves. There was a co-ordinating agent among matter, energy and force, which was one of the reasons responsible to manifest the life.

P1.29 continued

Aurbindo, Compiled by P.B. Saint - Hilaire, 1963 - "*The future evolution of man*" P. 50. Published by Shri Aurobindo Ashram, Publication, dpt. Pondicherry, INDIA.

Chyba, C.F., et.al. 1990; Science **249** : 366 - 373.

Deamer D.W., **June, 1997** : Microbiology and Molecular Biology Review P. 239 - 261.

Johan horgan, 1991; Scientific American, Feb - 1991 P: 116-128.

Miller, S.L., et.al. 1974, *`The Origin of Life on the Earth'* Prentice - Hall, Inc. Englewood Cliffs. N.J.

Miller, S.L., et.al. 1959; Science **130** : 245 - 251.

Oro J., et.al. P. 237 - 295. In R.P. Mortlock (ed.), The evolution of metabolic function CRC. Press, Inc., Boca Raton Fla.

Oparin A.I., 1924 *The Origin of Life*, Izd. Moskovshii Rabochii, Moscow, Russia, English Translation in J.D. Bernel 1967, *The origin of life* P. 199 - 234, Weidenfeld & Nicolson, London U.K.

Orgel L.E., 1994; Scientific American, Oct 1994, P: 77 - 83.

Wächtershäuser, G, 1988; Microbiol. Rev. **52** : 452 - 484.

P1.33

A COMPRENSIVE ORIGIN OF LIFE SCENARIO

Noam Lahav[1], Shlomo Nir[1], and Avshalom Elitzur[2]
[1]The Hebrew University of Jerusalem, Israel
[2]Bar-Ilan University, Israel

The coevolution theory (Lahav and Nir, 1997; Nir and Lahav, 1997) focuses on the emergence of template-and sequence-directed (TSD) synthesis in a prebiotic fluctuating environment, where the templates are small proto-RNA molecules.

A central assumption in the coevolution theory is the formation of a population of short and initially non-directed peptides; some of these can catalyze the reactions of the proposed scenario. The short catalytic peptides with their crude shape recognition are central to both a takeover process by catalytic TSD peptides, and their involvement in chiral selectivity. The resulting TSD systems are characterized by continuously evolving autocatalysis, feedback loops, a primordial translation system and its genetic code, as well as the ability to form and incorporate into their primordial "memory" novel functions, such as homochirality takeover and metabolic cycles. The biogeochemical systems distinguished by these features consist of dynamic populations of chemical entities characterized by the above primordial features. Some of these entities are assumed to have evolved into the populations of primitive cells which eventually constituted the last common ancestor. The advantage of this scenario is that, with a few plausible assumptions, the system's complexity and information capacity naturally follow.

Recent developments in the search for both early stages in the phylogenetic tree (Trifonov and Bettecken, 1997; the top-down strategy), and the transition from inanimate to animate according to the coevolution theory (the bottom-up strategy), suggest the merging of the evolutionary pathways explored by these approaches into a coherent and continuous scenario. The coevolution theory is essentially a simplification of central processes of extant living forms, which focuses on the emergence of TSD syntheses in compartmentalized entities in a prebiotic fluctuating (hydration-dehydration) environment. Thus, the transition from inanimate to animate is intellectually comprehensible and amenable for experimental exploration. In the comprehensive scenario which is based on both the bottom-up and top-down research strategies, this stage is followed by a transition from primordial entities characterized by a few

central attributes of life, to more evolutionary-advanced entities. For instance, during the latter transition the primordial genetic code of the bottom-up strategy evolved into the beginning of the modern genetic code suggested by the top-down strategy.

We have initiated the experimental program by means of a computer model (Nir an Lahav, 1997). However, in the absence of directly-relevant experimental data regarding short catalytic peptides, we are analyzing and using published molecular-biological kinetic data of the central reactions of our scenario, namely, template replication, amino acid loading onto proto-tRNA and peptide bond formation.

Lahav, N. and Nir, S.: 1997, Origins of Life Evol. Biosphere **27**, 377.
Nir, S. and Lahav, N.: 1997, Origins of Life Evol. Biosphere **27**, 567.
Trifonov, E.N. and Bettecken, T.: 1997, Gene **205**, 1.

P1.34

COMPARATIVE TRACE ORGANIC ANALYSIS OF HCN POLYMERIZATION MIXTURES AND A THOLIN

S.A. Liebman, The CECON Group, Inc.
242 N. James St., Wilmington, DE 19804;
R.A. Pesce-Rodriguez, M.A. Schroeder, U.S. Army Research Laboratory, Aberdeen Proving Ground, MD 21105;
C.N. Matthews, University of Illinois, Chicago, IL 60607.

The composition of hydrogen cyanide polymerization products is acknowledged to be significant data in understanding prebiotic and extraterrestrial chemistry. Continuing our analytical screening studies for complex mixtures of organic compounds from HCN polymerizations according to the analytical strategy initially reported in 1995(1), we now report comparisons with a Titan tholin sample synthesized by B. Khare via plasma discharge through a 90:10 mixture of N_2:CH_4, as described by Ehrenfreund, *et al* (2).

Importantly, our new nondestructive analysis employing X-band (9 GHz) electron spin resonance (ESR) spectroscopy provided astounding results. HCN polymerization mixtures that had been stored at room temperature for over eight years, as well as the more recent (ca. 2 year-old sample) Titan tholin (B. Khare) gave strong ESR signals for evidence of C- and N-based free radicals, the latter more evident in the tholin sample (Fig. 1). Future studies by Budil et al (3,4) using a unique 220 GHz ESR system will be used to resolve and identify the long-lived free radicals in HCN polymerizations and tholin mixtures.

Our earlier report (1) described the nondestructive analyses (extraction and chromatographic separations) and fragmentation techniques, including time-resolved pyrolysis direct insertion Pyroprobe (DIP)-mass spectrometry (MS) used for various HCN mixtures and reference compounds. Further information is now reported from study of supercritical fluid extraction (SFE) profiles; SFE extracts with off-line Fourier transform-infrared (FT-IR) microscopy; *in situ* monitoring of HCN polymerizations with fiber optic mid-FTIR; and particle beam liquid chromatography (PBLC)-MS. Other data from resonance Raman spectroscopy obtained in the early 1990s further support this new direct evidence of free radicals in solid HCN polymer samples.

P1.34 continued

In general, these analytical screening studies give compelling evidence for the presence of amino acids, nitriles, amides, N-heterocycles, and peptide precursors from gas phase and liquid HCN polymerization reactions. The Titan tholin mixture gave direct analytical similarities to HCN mixtures, plus evidence for hydrocarbon presence. Both HCN and tholin mixtures give refractory polymeric C,H,N network residuals which were stable to >1300 C.

We conclude that complex compositions of HCN multimers and polymers are also present in tholin mixtures, together with hydrocarbon species. The presence of long-lived free radicals in both HCN and tholin mixtures opens new energetic pathways that can provide wide-ranging functionalization of these prebiotic polymeric materials. Simulations using selected solid state reactions will now include the range of chemical dynamics inherent to free radical species.

(1) Liebman, S.A., Pesce-Rodriguez, R.A., and Matthews, C.N.: 1995, Adv. Space Res. **15**, 71-80.
(2) Ehrenfreund, P., Boon, J.J., Commandeur, J., Sagan, C., Thompson, W.R., and Khare, B.: Ibid., 335-342.
(3) Budil, D.E., Earle, K., Lynch, W., Freed, J.J.: 1989, in *Adv. EPR: Applications in Biology and Biochemistry*, A.J. Hoff, ed., 307-338.
(4) Cardin, J.T., Anderson, J.R., Kolaczkowski, S.V., and Budil, D.E.: 1999, Appl. Magn. Reson., in press.

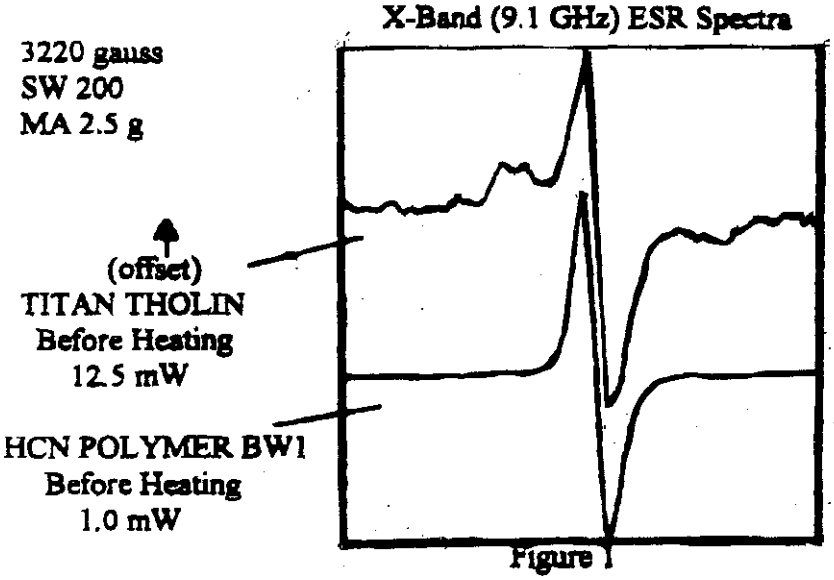


Figure 1

P1.35

SYNTHESIS FROM THIOGLUTAMIC ACID

Marie-Christine Maurel[#] and Leslie E. Orgel[*]
[#]Institut Jacques Monod, Tour 43, 2 place Jussieu, 75251 Paris cedex 05 France
[*]The Salk Institute for Biological Studies, P.O Box 85800, San Diego, CA, 92186

A central problem in origins of life studies is concerned with the prebiotic synthesis of peptides in aqueous environments. Numerous non prebiotic condensing agents, for example water-soluble carbodiimides and carbonyldiimidazole, have been used in the direct oligomerization of amino acids. In other studies pre-activated monomers have been used. Glycine thioesters, for example, have been shown to give short oligoglycines in good yields (Weber and Orgel, 1979). The relevance of thioesters to prebiotic chemistry has been emphasized recently (de Duve, 1991) in part because of interest in the deep sea vents (Holm et al, 1992; Huber and Wächtershauser, 1998).

Thioacids are good acylating agent in presence of an oxidizing agent (Liu and Orgel, 1997). Here, we discuss the elongation of $(Glu)_{10}$ by thioglutamic acid (GluSH) and the direct oligomerization of GluSH in the presence of various combinations of bicarbonate and ferricyanide. The extension of $(Glu)_{10}$ by (GluSH) in the absence of bicarbonate or ferricyanide is very slow because the thioacid at neutral pH is a very poor electrophile. A marked acceleration of the reaction in the presence of the bicarbonate is due to the formation of a N-carboxyanhydride which is an efficient acylating agent. The absence of elongation in the presence of ferricyanide when no bicarbonate is added is due to a very rapid cyclization reaction which competes with elongation and leads to the formation of a cyclic dipeptide. Finally, we find that in the presence of both bicarbonate and ferricyanide, substantial yield of $(Glu)_{11}$ are formed immediately after mixing and a slower reaction leads to the formation of oligomers up to at least 15mer. The efficiency of direct oligomerization of GluSH, parallels the efficiency observed for the elongation of a $(Glu)_{10}$ primer. When bicarbonate and ferricyanide are present, the main products obtained are oligoglutamic acids up to at least the 7mer.

de Duve, C.: 1991, *Blueprint for a cell : The Nature and Origin of Life,* Neil Patterson Publishers, Burlington, North Carolina.
Holm, N. G. et al. Origins Life Evol. Biosphere, 1992, **22**, Special Issue.
Huber, C. and Wächtershauser, G.: 1998, Science, **276**, 245-247.
Liu, R. and Orgel, L. E.: 1997, Nature, **389**, 52-54.
Weber, A. and Orgel, L. E.: 1979, J. Mol. Evol., **13**, 193-202.

P1.36

NITROGEN FIXATION BY FERROUS ION

David Mauzerall Rockefeller University, New York. NY 10021

The requirement for reduced nitrogen for living processes is as important as that for reduced carbon. Yet less research has been done on the fixation of nitrogen under prebiotic conditions than on the reduction of carbon dioxide. Earlier work by many groups have shown that ferrous ion near neutral pH is a powerful reductant, forming hydrogen in the dark and even more readily when photoexcited. Following on the work of Schrauzer and Guth (1976) who claimed that nitrogen was reduced to ammonia by ferrous ion specifically at pH 8.6, we have begun a study of both the dark and photochemical aspects of this intriguing reaction. We are determining the ammonia colorimetrically and will study the pH range of this reaction. We have found that the reaction may be photochemically driven by light of wavelength >320nm. Since such light is plentiful in solar radiation, the reaction could have occurred under anaerobic, prebiotic conditions where the ferrous ion was present.

Schrauzer, G. N. and Guth, T. D. :1976, J. Am. Chem. Soc. **98**, 3508-3513.

P1.37

ABIOTIC SYNTHESIS OF PYRIMIDINES FROM SIMULATED PRIMITIVE ATMOSPHERE

Ken-ichi Murasawa, Toru Tsuji, Shin Miyakawa* and Kensei Kobayashi
Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan
* Materials and Structures Laboratory, Tokyo Institute of Technology, Yokohama 226-8503, Japan

The discovery of ribozymes has led to formulation of "the RNA world" hypothesis. The question then arises as to the origin of RNA molecules on the primitive earth. If RNA molecules could not be formed on primitive Earth or on other bodies, the RNA world hypothesis would be analogous to a house built on sand. We examined the possibility of forming RNA bases from a simulated primitive atmospheres.

In a glass tube, 350 Torr each of CO and N_2 (or NH_3) was introduced. In some experiments, liquid water (10 mL) or water vapor (20 Torr) was also added. The gas mixture was irradiated with 2.0 mC of 3.0 MeV protons from a van de Graaff accelerator (Tokyo Institute of Technology). The irradiation product was acid-hydrolyzed, then was separated into two fractions with cation exchange resin AG-50WX8. Anionic/neutral species including uracil and thymine should be in the first fraction, and cationic species including cytosine and purine bases in the second. Each fraction was subjected to HPLC separation, desalting and derivatization with BSA, followed by GC/MS. Stable isotope labelled $^{13}C^{16}O$ purchased from ISOTEC was used in some runs to prove indigenousness of the product.

Uracil was identified in all the products. G-Values were in the range of 3×10^{-6} to 1×10^{-4}. Cytosine was also identified in the following system: CO and NH_3 ($G = 2\times10^{-5}$); CO, NH_3 and H_2O vapor ($G = 5\times 10^{-5}$); CO, N_2 and H_2O vapor ($G = 2\times10^{-5}$). It is suggested that the presence of NH_3 in the irradiation targets is required to form cytosine. No other bases - thymine and purine bases - were detected in any samples.

The present study suggests that only uracil could be formed in primitive atmosphere. Cytosine may be formed in interstellar dust grain environment where NH_3 is believed to be present with CO and H_2O. It is essential to find out possible formation pathway of purines for the prerequisite of the RNA world.

P1.38

FORMATION OF "PROTO-ESTERASES" FROM SIMULATED PRIMITIVE ATMOSPHERES BY RADIATION

Tomohiro Tsuruda, Tomoko Takahashi, Yukihisa Funatsu
and Kensei Kobayashi
Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan

Proteins, together with a kind of RNAs, have a catalytic roles in the present living systems. It has not proved, however, that these macromolecules, peptides or oligonucleotides are formed under plausible primitive planetary conditions.

We reported that a mixture of complex organic compounds whose average molecular weight was some hundred was formed when a mixture of carbon monoxide, nitrogen and water was irradiated with high energy protons (Kobayashi et al., 1997). This "complex organics" gave amino acids after hydrolysis. We examined whether the complex organics have catalytic activity to hydrolyze esters.

A gas mixture of CO and N_2 (350 Torr each) was enclosed in a glass tube with 20 mL of water and was irradiated with 3.0 MeV protons from a van de Graaff accelerator (TIT, Tokyo). The esterase activity was assayed by flow injection analysis with fluorescein diacetate as a substrate.

The irradiation product showed esterase activity: When energy of 3.5 kJ was deposited in the gas mixture, the whole product showed the activity of 107 μU at pH 7.5 (1 U = 1 μmol min^{-1} at 30°C). When the product was mildly hydrolyzed with 1 M HCl at 110°C, the activity increased by 30% for the first 20 min, then it started to decrease. When the product was separated into seven fractions (A-G) by cation-exchange chromatography, Fraction B near void volume showed the maximum activity, followed by Fraction G. Imidazole was detected in the product, was known to have esterase activity, and eluted in the Fraction G. It was shown that compounds with esterase activity, other than imidazole, can be abiotically formed from CO, N_2 and H_2O by radiation.

It is of interest to identify the compounds with esterase activity, and to examine whether they hydrolyze the primordial soup to increase themselves. Such works are in progress.

Kobayashi, K., Sato, T., Kajishima, S., Kaneko, T., Ishikawa, Y. and Saito, T.: 1997, Adv. Space Res. **19**, 1067.

P1.39

MECHANISMS OF AMINO ACID FORMATION IN SIMULATED PRIMITIVE ATMOSPHERES BY RADIATION

Kentaro Ushio, Takeo Kaneko, Koshi Kato, Takumi Kawase,
Takeshi Saito* and Kensei Kobayashi
Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan
* ICRR, University of Tokyo, Tanashi 188-8502, Japan

From a strongly reduced-type gas mixtures containing methane, amino acids can be formed easily by spark discharges and by radiation. If a gas mixture with carbon monoxide as a carbon source, however, amino acids can be formed by radiation much easily than by spark discharge. We analyzed possible intermediates of amino acid formation either by radiation or by spark discharge, and discuss possible formation pathways of amino acids in each case.

A gas mixture of CO (or CH_4) and N_2 (350 Torr each) was enclosed in a glass tube with 20 mL of water. The gas mixture was irradiated with 3.0 MeV protons from a van de Graaff accelerator (Tokyo Institute of Technology). The same kind of the starting mixture was subjected to spark discharge by using Tesla coil. Gasous products were determined by GC and/or GC/MS. The following were determined in the aqueous products: (i) Free HCN (colorimetry); (ii) total CN (ion-selective electrode), (iii) free aldehydes (by reversed-phase HPLC), (iv) NH_3 (colorimetry). Amino acids were determined by an amino acid analyzer after acid hydrolysis.

In the case of spark discharge in the CH_4 system, free CN and total CN was increased soon after initiation. After a while, total CN began to decrease, then yield of amino acids increased. The CO system gave much less amount of each product than the CH_4 system. It is suggested that cyanides in the solution are important intermediates of amino acids in discharge synthesis.

In the case of proton irradiation of both the CO system and the CH_4 system, however, yields of free CN, total CN, aldehydes, NH_3 and amino acids are proportional to the deposited total energy. Yields of amino acids are of the same order as that of cyanides, aldehydes or hydrocarbons. It is strongly suggested that amino acid precursors of large molecular weights were formed directly from the gaseous components, not from aqueous intermediates.

P1.40

Structural Investigations of Hydrogen Cyanide Polymer – (III) Comparison of TMAH thermochemolysis products from HCN polymer and N_2/CH_4 tholin; (IV) On-line ESI-MS analysis of HCN oligomerization.

Robert Minard, Kim Kahle, Emily Stauffer, and Vanessa Amme, Penn State University, University Park, PA 16802, USA and Clifford Matthews, Univ. of Illinois, Chicago, Illinois, 60680, USA

Hydrogen cyanide polymers form spontaneously from HCN and traces of base catalysts. It is probable that these dark brown/black materials played an important role in the early stages of chemical evolution. Nevertheless, their full structural characterization has still not been accomplished.

Tholin, produced from plasma discharge through a 90:10 mixture of N_2/CH_4 in studies designed to simulate production of aerosol detected in the Titanian atmosphere, also contain dark-colored, high molecular weight materials(1).

Heating complex heteropolymers in sealed tubes with tetramethylammonium hydroxide to 300°C for 30 min (TMAH thermochemolysis) causes bond cleavage and in situ methylation producing a suite of products which can be analyzed by GC-MS yielding valuable insight into the substructural features of the parent polymers.(2)

TMAH thermochemolysis/GC-MS of hydrogen cyanide polymers reveals a complex suite of products including methylated amino acids and nitrogen heterocycles, such as purines, pyrimidines, and triazines, providing new insight into the substructural motifs that are present in HCN polymers.(3) Recent work verified structural assignments and identified additional compounds. Application of this analytical method to tholin allowed comparison of this material and HCN polymer. Nineteen of the twenty-five TMAH thermochemolysis products found in HCN polymer were also derived from the tholin sample providing strong evidence that tholin contains a substantial amount of HCN polymer. (see Table 1)

Flow injection electrospray MS analysis can be used to study the base-catalyzed HCN polymerization in real time providing structural information on the initially formed products. A refined understanding of these initial oligomerization steps provides insight into the structure of HCN polymer.

P1.40 continued

Structural Investigations of Hydrogen Cyanide Polymer, Minard et al.

Table 1 Products from HCN polymer and Titan tholin.

	Methylated Derivative (MW)	Parent Structure	$(HCN)_x$	Tholin
1	N,N-Dimethylaminoacetonitrile	Aminoacetonitrile	+	?
2	N-Methylformamide	Formamide	+	?
3	N,N-Dimethylcyanamide	Cyanamide	+	+
4	N,N-Dimethylformamide	Formamide	+	+
5	N-Methylcarbamic Acid, methyl ester	Carbamic Acid	+	+
6	N,N-Dimethylcarbamic acid, methyl ester	Carbamic Acid	+	+
7	N-Methylacetamide	Acetamide	+	+
8	N,N-Dimethylglycine, methyl ester	Glycine	+	+
8a	N-Methylisobutyramide	Isobutyramide	+	?
9	N,N-Dimethylacetamide	Acetamide	+	+
10	N,N'-Dimethylalanine, methyl ester?	Alanine?	?	?
12	Succinic Acid, dimethyl ester	Succinic Acid	+	+
13	N,N,N'-Trimethylurea	Urea	+	trace
14	N-Methylsuccinimide	Succinimide	+	+
15	Methylglutarimide?	Glutarimide?	+	+
17	Glutaric Acid, dimethyl ester	Glutaric Acid	+	?
18	Methyldihydrouracil? or Dihydrothymine?	Dihydrouracil or Dihydrothymine	+	+
19	Dimethyldihydrouracil? or Methyldihydothymine?	Dihydrouracil or Dihydrothymine	+	+
20	N,N,N-Trimethyl-sym-triazinetrione	"sym-Triazine"	+	++
21	N,N-Dimethyluracil	Uracil	+	+
22	N,N,N-Trimethyl-sym-Triazinedioneimine	"sym-Triazine"	+	trace
23	N,N,N-Trimethyladenine	Adenine	+	trace
24	Caffeine	Xanthine	+	+

(1) Ehrenfreund, P. Boon, J.J., Commandeur, J. Sagan, C., Thompson, W.R., Khare, B., 1995, Analytical Pyrolysis Experiments of Titan Aerosol Analogues in Preparation for the Cassini/Huygens Mission, *Adv. Space Res.*, 15, 335-342 and references cited therein.
(2)McKinney, D.E., Carson, D.M., Clifford, D.J., Minard, R.D. and Hatcher, P.G. 1995. Off-line thermochemolysis versus flash pyrolysis for the in situ methylation of lignin: is pyrolysis necessary?, *J. Anal. & App. Pyrolysis*, 34: 41-46.
(3) Minard, R.D., Hatcher, P.G., Gourley, C.R., and Matthews, C.N. 1998. Structural Investigations of Hydrogen Cyanide Polymers: New Insights Using TMAH Thermochemolysis/GC-MS, *Origins of Life and the Evol. of the Biosphere*, 28, 461-473.

P1.41

THE RÔLE OF CARBON MONOXIDE IN POSSIBLE PREBIOTIC REACTIONS

Gyula Pályi and Claudia Zucchi
Department of Chemistry, University of Modena, V. Campi, 183, I-41100 Modena, Italy

Geologic records of conditions in atmosphere and hydrosphere of early Earth are almost completely lacking. Indirect evidence [1] indicates large quantities of N_2 and CO_2, together with minor amounts of CO, CH_4, water vapour, H_2, H_2S, SO_2. Atmospheric total pressure can reach initially 60 bar which later drops to 10-20 bar. Surface temperature is supposed to be 30-50 °C, in vulcanic/hydrothermal areas much higher (100-500°C). Low valent transition metals (primarily Fe) and low valent forms of S (perhaps also Se) are available.

Under these conditions a very rich chemistry on the basis of CO, assisted and/or catalyzed by transition metal carbonyls can develop [2]. It is the goal of the present paper to analyze the possibilities of

- steam reforming ($C + H_2O \rightarrow CO + H_2$)
- water gas shift ($C + H_2O \rightarrow CO_2 + H_2$)
- CO hydrogenation ($C + H_2 \rightarrow HCHO$
  ($C + H_2 \rightarrow (CH_2)_x$ - OH)
  ($C + H_2 \rightarrow (CH_2)_x$ etc.)
- hydroformylation (>=< + CO + H_2 → aldehyde)
- hydroxycarbonylation (>=< + CO + H_2O → carboxylic acid)
- amidocarbonylation (>=< + CO_2 + NH_3 → amino acids)
- CO protonation by Brønsted acidic mineral surfaces (e.g. → HCHO, etc.).

It is concluded that these reactions could provide an important contribution to the variety of prebiotic organic compounds on the early Earth.

[1] Bengtson, S. (Ed.) 1994, *Early Life on Earth*, Columbia University Press, New York.
[2] Cornils, B. and Herrmann W.A. (Eds.): 1996, *Applied Homogeneous Catalysis with Organometallic Compounds*, VCH, Weinheim.

P1.42

TEACHING ON THE ORIGIN OF LIFE TO UNDERGRADUATE STUDENTS AT THE UNIVERSITY OF VALENCIA

Juli Peretó
Departament de Bioquímica i Biologia Molecular, Universitat de València, E-46100 Burjassot (Spain)

In the academic years 97-98 and 98-99, the Department of Biochemistry and Molecular Biology of the University of València has offered an undergraduate course for students with a Biology or Biochemistry major entitled "Chemical and Biochemical Evolution". During a semester this course of two 1h-lecture per week presents topics on chemical evolution (including the origin of the elements and the solar system, prebiotic organic chemistry, and prebiotic selection) and biochemical evolution (the origin of membranes and bioenergetic processes, the origin of genetic information, the development of metabolic pathways). In a parallel series of ten 2h-sessions of group discussion, the historical context of the problem is presented by reading original texts by Pasteur, Oparin, and Haldane; research papers on the ribonucleic nature of peptidyl transferase (to discuss the hypothesis of an RNA world) and on genome sequencing data from bacteria and mitochondria (to discuss the symbiogenetic origin of eukaryotic organelles) are also presented. Some videotapes on star evolution, simulation of impacts on the arcaic Earth, the high- vs. low-temperature origins debate or on some experimental approaches in prebiotic chemistry are also discussed in group. Finally the students simulate selective processes using a computer program and also are introduced to the use of interesting web sites in Internet. Students must also present at the end of the semester an assay on a book chosen between different topics related to the course, e.g., artificial life, microbial evolution or virus evolution.

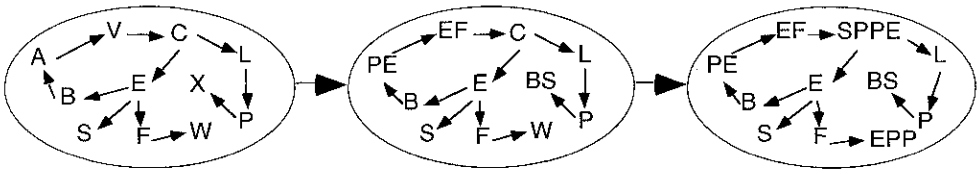
The aim of this communication is to discuss with other collegues already teaching on the origin of life at their university or planning to do it in the next future. The development of most of these teaching activities were possible with the invaluable help and encouragement of Prof. Antonio Lazcano (UNAM, México), Visiting Professor at the University of València. The simulation program was developed by the Group of Biophysics at the Complutense University (Madrid, Spain) under the supervision of Prof. Federico Morán.

P1.43

THE PREBIOTIC TRANSITION FROM COMPOSITIONAL TO SEQUENCE-BASED INFORMATION

Daniel Segré, Dafna Ben-Eli and Doron Lancet,
Dept. of Molecular Genetics and the Genome Center, the Weizmann Institute of Science, 76100 Rehovot, Israel. E-mail: bmsegre@wicc.weizmann.ac.il

Some origin of life scenarios envisage primitive forms of reproduction, even in the absence of self-replicating informational biopolymers (reviewed in Segre and Lancet, 1999). Such systems may be described in terms of chemical kinetics and physical properties, thus allowing one to understand their spontaneous and gradual emergence from inanimate chemical mixtures. Within this framework, we have suggested that the first biological information might have been mainly compositional (Segré and Lancet, 1999, Segre et al. 1999). This, however, results in a challenging open question: what was the path from such early stages to a genetic code, based on "alphabetic" information storage? Here we suggest a possible scenario for this transition.



Within an efficient mutually catalytic assembly of monomeric units, as described by the Graded Autocatalysis Replication Domain (GARD) model (Segré et al., 1998), dimers and higher oligomers would gradually form. These could replace some of the monomers, assuming their catalytic roles in the network. We show that rearrangements of monomers within the oligomers would make the new assemblies more successful in propagating their "compositional genome". In addition to this selective advantage, oligomers could also exhibit higher mutually catalytic potencies because of a "combinatorial library" effect. Our computer model utilizes a fitness function that balances the thermodynamic price of polymerization with the advantages of oligomer formation. In preliminary simulations, a defined size of monomer alphabet was reached and a hierarchy of oligomer "words" crystallized. This simulated process may help understand the appearance of chemical combinatorics, a prerequisite for the emergence of a genetic code.

Segré, D., Lancet, D., Kedem, O. and Pilpel, Y.: 1998, Orig. Life Evol. Biosphere, **28**, 501-514.
Segré, D., Lancet, D.: 1999, Chemtracts - Biochem. and Mol. Biol., 12(6).
Lancet, D., Ben-Eli, D. Deamer, D. and Segré, D., ISSOL 1999.

P1.45

FORMATION OF CARBOXYLIC ACIDS BY UV IRRADIATION OF MIXTURES OF SIMPLE CARBON COMPOUNDS AND WATER AT ROOM TEMPERATURE AND 77 K

Akira Shimoyama, Yasuhisa Morita, Yoshinori Takano, Shinki Terada, and Hajime Mita
Department of Chemistry, University of Tsukuba, Tsukuba 305-8571, JAPAN

We carried out UV irradiation experiments of mixtures of a simple carbon compoud, such as benzene, acetaldehyde or graphite, and water at room temperature (R.T.) and 77 K, using a low pressure mercury lamp (254 nm wave length) and a Xe eximer lamp (172 nm wave length). The irradiation products were analyzed for monocarboxylic acids (MCAs) and dicarboxylic acids (DCAs). The MCAs were analyzed directly and DCAs were analyzed after acylation by a gas chromatograph combined with a mass spectrometer. Identification and quantification of the MCAs and DCAs in products were made by a comparison with standard compounds.

The results of irradiation of the mixture of benzene and water showed the formation of C_2 to C_6 MCAs and C_2 to C_8 DCAs by 254 nm at R.T. at the yield levels 0.003 to 0.3% on the basis of carbon atoms with higher yields of DCAs than those of MCAs. At 77 K, only acetic acid and C_2 to C_5 DCAs were formed by 254 nm.

Irradiation of the mixture of acetaldehyde and water by 172 nm at R.T. yielded a total of 7 MCAs from C_2 to C_6 consisting of 5 normal and 2 branched structures, and 22 DCAs from C_2 to C_7 of normal, branched and unsaturated structures. The total yield of MCAs and DCAs was 0.2%. At 77 K, 6 MCAs from C_2 to C_5 and 16 DCAs from C_2 to C_6 were formed with the total yield 0.06%.

The mixture of graphite and water yielded 11 MCAs from C_2 to C_6 with 0.006% yield by 172 nm at R.T., and mainly acetic acid at 77 K with much smaller yield than that at R.T.

These results of the UV irradiations were used to evaluate the quantities of carboxylic acids formed on the primitive earth and on the surface of comets using quanta of light irradiated in a view of chemical evolution.

P1.46

AN ECOSYSTEM MODEL FOR PREDICTION OF METHANE FLUX INTO THE ARCHEAN ATMOSPHERE

Janet L. Siefert[1] and James F. Kasting[2]
[1]Dept of Statistics, Rice University, Houston, TX 77251-3891 and [2]Dept of Geosciences, PSU, State College, PA 16802

Atmospheric methane may have played a significant role in warming the surface of early Earth. Photochemical calculations suggest that a CH_4 flux comparable to the present biological flux could have produced methane mixing ratios on the order of 10^{-3} -- high enough to offset low solar luminosity even at today's low CO_2 level. Methane may also be a useful biomarker molecule in the atmospheres of early-Earth type extrasolar planets. For both these reasons, it would be useful to be able to estimate the methane flux on the early Earth. Abiotic sources of methane can be estimated by comparing with modern (submarine) volcanic fluxes. The biotic source is more difficult to compute, as it would have depended on the nature of the Archean ecosystem. Methanogens were most likely only one component of a diverse microbial ecosystem that evolved, continuously exploiting metabolic strategies in response to ongoing reductive and oxidative geologic and ecosystem processes. Sequence acquisition of prokaryotic genomes in their entirety provides the means for identifying lateral gene transfer and gene duplication events. This information can be used to reconstruct the evolutionary histories of metabolic pathways, setting time constraints on the various events relative to their appearance in the molecular record. One can then use this information to model the nature of the dominant microbial community during periods of ecosystem transition as indicated by the geologic record. We describe an integrated approach to evaluating the Archean biosphere through i) computer modeling of the early environment, ii) phylogenetic reconstruction of genes and pathways for major contributing species of extinct and/or extant C_1 metabolizing microbes and iii) a mixed-effect modeling strategy to predict microbial interactions in flux with an ecosystem.

P1.48

PREBIOTIC NITROGEN FIXATION BY FES REDUCTION OF NITRITE UNDER ACIDIC CONDITIONS

David P. Summers, SETI Institute
NASA - Ames Research Center, M/S 239-4, Moffett Field, CA 94035-1000

Theories for the origin of life require the availability of reduced nitrogen for the formation of such species as amino acid and nucleic acids. Initial theories supposed a reducing atmosphere of methane and ammonia in which compounds essential to the chemical evolution of life, such as amino acids, can form from spark discharges (Chang, S., DesMarias, D. et al., 1983; Stribling, R. E. and Miller, S. L., 1987). However, current geochemical evidence points to a non-reducing atmosphere, made primarily of carbon dixoide and nitrogen, in which electric discharges produced NO and CO (Yung, Y. L. and McElroy, M. B., 1979; Chameides, W. L. and Walker, J. C. G., 1981; Fegley Jr., B., Prinn, R. G. et al., 1986; Kasting, J. F., 1990). This raises the questions of; how can ammonia be formed under a neutral atmosphere, and what conditions are needed such formation to occur?

One possibility is the conversion of NO into nitric and nitrous acids (through HNO) which is rained into the oceans (Mancinelli, R. L. and McKay, C. P., 1988). The reduction of nitrite by aqueous Fe(II) such as was present on the early Earth (Holland, H. D., 1973; Holland, H. D., 1984; Walker, J. C. G. and Brimblecombe, P., 1985) could then have produced ammonia (Summers, D. P. and Chang, S., 1993). However, this

$$6\ Fe(II) + 7\ H^+ + NO_2^- \rightarrow 6\ Fe(III) + 2\ H_2O + NH_3 \qquad (1)$$

reaction does not proceed at a pH of less than 7.3. An alternative is reduction by other forms of Fe(II), such as FeS. We will present results that show that FeS can reduce nitrite to ammonia at pHs as low as pH 5 under a variety of conditions.

The reaction of nitrite proceeds well with FeS. The concentration of nitrite shows a reasonable first order decay, consistent with the rate law seen for reduction by Fe^{+2} (Summers, D. P. and Chang, S., 1993). At room temperature, under CO_2, at pH 6.25, and with 9.4 mg/ml FeS, a reaction rate of $k = 2.4 \times 10^{-4}$ min • g/l was measured. The product yield (%age of nitrite converted to ammonia) was 27%. The reaction runs under CO_2 from pH 4.9 to pH 7.2. Over this range product yield decreases from 53% at pH 5.0 to 18% at pH 6.9. The reaction should be favored by lower pH but presumably the increasing concentration of bicarbonate interferes with the reaction.

P1.48 continued

The reaction proceeds well in presence of such species as chloride, sulfate, and phosphate. With chloride and sulfate, yields dropped from 29% to 25%. With phosphate yields dropped to 11%. Comparing the reaction in a phosphate buffer under CO_2 vs one under N_2 showed a lower yield under CO_2 (11% vs 20%) showing that bicarbonate does indeed have a detrimental effect.

Chameides, W. L. & Walker, J. C. G.: 1981, Origins Life **11**, 291-302.
Chang, S., DesMarias, D., Mack, R., Miller, S. L., & Strathearn, G. E.: 1983, *Earth's Earliest Biosphere: Its Origin and Evolution*, Princeton University Press, Princeton, NJ, p. 53-92.
Fegley Jr., B., Prinn, R. G., Hartman, H., & Watkins, G. H.: 1986, Nature **319**, 305-308.
Holland, H. D.: 1973, Econ. Geol. **68**, 1169-1172.
Holland, H. D.: 1984, , Princeton University Press, Princeton, NJ, p. 387-388 & 396-397.
Kasting, J. F.: 1990, Origins Life Evol. Biosphere **20**, 199-231.
Mancinelli, R. L. & McKay, C. P.: 1988, Origins Life Evol. Biosphere **18**, 311-325.
Stribling, R. E. & Miller, S. L.: 1987, Origins Life **17**, 261-273.
Summers, D. P. & Chang, S.: 1993, Nature **365**, 630-633.
Walker, J. C. G. & Brimblecombe, P.: 1985, Precambrian. Res. **28**, 205-222.
Yung, Y. L. & McElroy, M. B.: 1979, Science **203**, 1002-1004.

P1.49

N-CARBAMOYLAMINOACID SOLIG-GAS NITROSATION NO/NO_x : A NEW ROUTE TO OLIGOPEPTIDES VIA AMINOACID N-CARBOXYANHYDRIDE. PREBIOTIC IMPLICATIONS.

Jacques Taillades*, Hélène Collet, Laurence Garrel, Isabelle Beuzelin, Laurent Boiteau, Henri Choukroun, Auguste Commeyras

Organisation Moléculaire – Evolution et Matériaux Fluorés - Chemistry Department (CC 017), University of Montpellier II, Place E. Bataillon, 34095 Montpellier cedex 5, France. *e-mail* acommeyras@crit.univ-montp2.fr

The synthesis of macromolecules such as RNA or peptides in prebiotic conditions is still an open problem. We have previously shown that in such conditions, α-N-carbamoylaminoacid (CAA) (rather than free α-aminoacids) are a convergent evolutionary end [Taillades *et al.* 1998].

Despite their high stability in a primitive hydrosphere, we have discovered that they may have been an important intermediate in prebiotic peptide synthesis, through activation by atmospheric NOx. Thus nitrosation of solid CAA (glycine or valine derivative) by $1/4O_2$/NO gaseous mixture (1 atm) yields quantitatively N-carboxyanhydride (NCA) in less than 1h at room temperature. In a prebiotic context, the efficiency of the nitrosation reaction depends on the O_2/NO ratio, with an optimum at 1/4 : at lower O_2/NO, NCA is still formed though in lower yield (with a linear dependance to O_2/NO).

The crude solid NCA undergoes quantitative oligomerization (from trimer to nonamer in the conditions we used) when treated by a (bi)carbonate aqueous buffer at pH 9, while complete hydrolysis occurs in pure water. Studies on the peptide elongation through such a repeated reaction cycle are in progress.

We therefore suggest that a part of the prebiotic amino acid activation/polymerization process may have taken place in a dry phase such as "drying-lagoon" scenario.

Collet, H., Bied, C., Taillades, J., Mion, L. Commeyras, A: 1996, Tetrahedron Letters **37**, p 9043-9046

Taillades, J., Beuzelin, I., Garrel, L., Tabacik, V., Bied, C. and Commeyras, A: 1998, Origins of Life and Evolution of the Biosphere, **28**, p 61-77

Taillades, J., Collet, H., Garrel, L., Beuzelin, I., Boiteau, L., Choukroun, H., Commeyras, A: 1999, Journal of Molecular Evolution (accepted).

P1.51

ALUMINA-CATALYZED AMINO ACID CONDENSATION: INFLUENCE OF SURFACE ACIDITY

Vladimir A. Basiuk, Juan Sainz-Rojas and Rafael Navarro-González
Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México, Circuito Exterior C.U., 04510 México, D.F., MEXICO

Amino acid condensation catalyzed by inorganic oxide surfaces is a widely recognized scenario for prebiotic peptide formation [1-8]. Previous simulation experiments normally involved different kinds of clays. The results strongly support the heterogeneous condensation hypothesis, but at the same time give poor insights into chemical mechanisms of the peptide formation. This is due to complexity of clays and related materials in terms of surface chemistry, i.e. due to the presence of different active surface sites, from single atoms and surface functional groups to planes, layers, edges, etc.

An approach, which our laboratory is developing for elucidation of the chemical mechanisms, is "splitting" complex oxide catalysts into simpler or elementary (when possible) oxide components. Thus, studying the catalytic properties of pure silica and alumina [9,10] should complement the experiments performed with clays: this should help to understand what surface active sites (or else surface atoms, i.e. Si or Al) might be responsible for the catalytic activity.

In continuation of the mechanistic studies of peptide bond formation catalyzed by simple inorganic oxides [9,10], in the present work we test catalytic activity of three different forms of alumina (neutral, basic and acidic) having different surface acidity, in the reaction of intermolecular condensation of L-alanine. For the purpose of correct comparison of the catalytic activity, all three forms had the same surface area, 155 m^2 g^{-1}; other reaction conditions were also maintained identical. Alumina samples were impregnated with alanine solutions and dried under ambient conditions, and then subjected to heating at temperatures up to 110 °C during several hours or days. No fluctuating drying/wetting conditions were simulated. Peptide products were quantified by means of high-performance liquid chromatography. It was found that under temeratures less than 100 °C, even after 5-day heating, the only detectable peptide product was L-Ala-L-Ala. We found strong dependence of its yields on the form of alumina employed: neutral alumina exhibited highest catalytic activity, followed by the basic form (roughly 1.5 times less active). Acidic alumina had activity roughly one order of magnitude less than the neutral form.

At 110 °C, besides dialanine, the only additional condensation product detected was related diketopiperazine [cyclo-(L-Ala)$_2$], with yields ca. 1 order of magnitude lower that those of the linear dipeptide. No racemization resulting in diastereomer formation was observed under the above conditions.

The results obtained are discussed in terms of surface chemistry.

P1.51 continued

REFERENCES

1. N. Lahav, D. White and S. Chang. *Science*, 1978, **201**, 67.
2. N. Lahav. *Heterogen. Chem. Rev.*, 1994, **1**, 159.
3. J.V. Smith. *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 3370.
4. J. Bujdak and B.M. Rode. *J. Mol. Evol.*, 1997, **45**, 457.
5. J. Bujdak and B.M. Rode. *React. Kinet. Catal. Lett.*, 1997, **62**, 281.
6. J. Bujdak and B.M. Rode. *J. Mol. Evol.*, 1996, **43**, 326.
7. K.I. Zamaraev, V.N. Romannikov, R.I. Salganik, W.A. Wlassoff, and V.V. Khramtsov. *Origins Life Evol. Biosphere*, 1997, **27**, 325.
8. T.L. Porter, M.P. Eastman, M.E. Hagerman, L.B. Price and R.F. Shand. *J. Mol. Evol.*, 1998, **47**, 373.
9. V.A. Basiuk, T.Yu. Gromovoy, V.G. Golovaty, and A.M. Glukhoy. *Origins Life Evol. Biosphere*, 1991, **20**, 483.
10. T.Yu. Gromovoy, V.A. Basiuk, and A.A. Chuiko. *Origins Life Evol. Biosphere*, 1991, **21**, 119.

cA1.1

METHANE IN THE ARCHEAN ATMOSPHERE

James F. Kasting
443 Deike, Penn State University, University Park, PA16802

There is still no consensus regarding the precise concentration of O_2 in the Archean atmosphere, but at least some authors agree that it was vanishingly low (Kasting, 1993, and references therein). If so, then methane may have been relatively abundant. Methanogenic bacteria, which produce most of Earth's methane today, are considered to be evolutionarily ancient (Woese and Fox, 1977). Furthermore, most methanogens can utilize hydrogen: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$, which should also have been relatively abundant in a low-O_2 atmosphere. Model calculations using present volcanic outgassing rates, and assuming diffusion-limited loss of hydrogen to space, predict atmospheric H_2 concentrations of ~0.1 percent, or 10^{-3} atm (Kasting, 1993). This is comparable to typical Michaelis-Menton parameters for H_2 uptake (Zinder, 1993), which suggests that methanogens should have thrived in the surface environment.

Photochemical calculations (Brown, 1999) indicate that a biological methane source equal to the present-day one could have produced Archean CH_4 mixing ratios on the order of 10^{-3}. Climate model calculations show that this amount of methane could have had a major warming effect on Earth's climate and may have been important in offsetting the effects of the faint young Sun. So far, though, no one has attempted to estimate the CH_4 flux that might be expected from a largely anaerobic Archean ecosystem. Preliminary efforts to do so are underway and will be discussed.

Brown, L. L.: 1999, *Photochemistry and Climate on Early Earth and Mars*, Ph.D. thesis, Penn State University.
Kasting, J. F.: 1993, Science **259**, 920.
Woese, C. R., & Fox, G. E.: 1977, Proc. Natl. Acad. Sci. USA **74**, 5088.
Zinder, S. H: 1993, In *Methanogenesis: Ecology, Physiology, Biochemistry, and Genetic*, ed. J. G. Ferry,. New York: Chapman and Hall, p. 128.

cA1.2

PRODUCTION OF REACTIVE NITROGEN IN EXPLOSIVE VOLCANIC CLOUDS

Rafael Navarro-González[1], Mario J. Molina[2] and Luisa T. Molina[2]
[1]*Laboratorio de Química de Plasmas y Estudios Planetarios, Instituto de Ciencias Nucleares, U.N.A.M., Cd. Univer., México D.F. 04510, MEXICO.*
[2]*Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge Massachusetts 02139, U.S.A.*

The production of reactive nitrogen species was essential for the synthesis of two classes of organic compounds which were likely the constituents of the first lifeforms on Earth, *e.g.,* amino acids and nucleic acid bases. Here we examine a novel mechanism to fix nitrogen in the early Earth by lightning discharges occurring inside explosive volcanic clouds (Navarro-González *et al.*, 1998). The chemical composition of gases emitted by primitive volcanoes is not known. We have assumed that it was similar to that of Hawaiian volcanoes, believed to arise from a primordial, undegassed reservoir deep in the Earth's mantle. Specifically the following composition was used: 50% H_2O, 30% CO_2, 11% N_2, 4.5% CO, and 4.5% H_2. Volcanic lightning was simulated in the laboratory using a hot and dense plasma produced by flowing the gas mixture (~1% in He) into a microwave discharge cavity where the gases were excited at 7 Torr and then the products were chemically ionized by He^+ produced in a glow discharge. The resultant ions were transported through an electrostatic ion guide operated at 10^{-4} Torr to a quadrupole mass spectrometer where they were quantified. Nitric oxide was found to be the major product formed. Its identity was further confirmed by infrared spectroscopy and by electron impact mass spectrometry of the individual product isolated by gas chromatography. We have estimated that about 5×10^{12} g of NO could have been produced annually by volcanic clouds occurring about 4 Gyr ago.

Navarro-González, R, Molina, M.J. and Molina, L.T.: *1998, Geophys. Res. Lett.* **25**, 3123.

cA1.3

OXYGEN AND OXIDIZING FREE-RADICALS IN THE HYDROSPHERE OF EARLY EARTH

Ivan G. Draganić

Institute of nuclear sciences Vinča, P.O.Box 522
11001 Beograd (Serbia),Yugoslavia

The liquid water is considered to be the quintessential environment criterion for chemical evolution processes leading to life. One of the benchmarks of Oparin´s idea on the origin of life was an anoxic primitive Earth and the studies of prebiotic chemistry do not take into account the possible presence of oxygen and oxidizing chemical species in the primeval hydrosphere. The potential factors in their production - cosmic and radioactive rays - are generally assumed to be of minor importance.

Radiation chemical approaches to chemical evolution suggest, however, that ionizing radiation can not be neglected as an energy source (Draganić,1999). It is certain that the amount of ionizing radiation energy was modest comparing to some other energy sources for chemistry on primitive earth (ultraviolet light, electric discharges) . Yet , the all over presence of radionuclides in specific environments may have provided significant amounts of energy on the geological time scale, like the potassium-40 throughout the ocean volume .

An important aspect of radiation chemistry is that the energy deposit and, subsequently, the origin of reactive species (ions, free-radicals, radical-ions) take place along the pathways of radiation regardless the physical state (gas, liquid, solid) or physical conditions (temperature, pressure) of the environment. This means that radiation-induced chemistry may take place in conditions hostile for chemistry that we know in laboratory(ocean depths, underground waters, cometary ice).

Molecular O_2 and H_2O_2 and various short-living species, among them the oxidizing radicals OH and HO_2 , appear in irradiated water . Reacting with solutes present in the bulk of water , these primary products of water radiolysis cause a large number of chemical reactions. For example, there are some sixty reactions in the sea

cA1.3 continued

water where, besides the products of pure water radiolysis, numerous reactive intermediates appear : Cl, Br, Cl_2^-, Br_2^-, $ClOH^-$, $BrOH^-$ and SO_4^-. Other oxidizing radicals such as CO_2^- and CO_3^-, in addition to oxidizing species known in the radiolysis of pure water, were present in sandstone underground waters flowing through the cores of natural nuclear reactors (Oklo phenomenon).

Computer simulations show that the amounts of radiation produced oxidizing species were modest in the primitive hydrosphere. In the early ocean the annual generations of free radicals and molecular products were on the picomolar and micromolar scales respectively. It is important to note that this generation of radiation-produced oxidizing species occurred homogeneously, uninterrupted, and undisturbed because of the nature of energy supply process,the radioactive decay of potassium-40. In underground waters, because of higher amount of energy deposited by radioactive nuclides from uranium fission process, concentrations were higher : micromolar and millimolar for the free-radicals and molecular products respectively.

Present radiation chemical experiments and computer modeling of radiation-induced processes in primitive hydrosphere suggest the intrinsic oxidizing capacity of the early hydrosphere . A more detailed insight into this phenomenon may be of interest for routine approaches to prebiotic chemistry.

Reference

Draganić, I.G. : Radiation chemical approaches to chemical evolution processes on earth and beyond. Chapter II in *The Role of Radiation in the Origin and Evolution of Life*, Kyoto University Press, Kyoto 1999 (in press).

cA1.4

CR OXYGEN BAROMETRY: OXIDATION STATE OF MANTLE-DERIVED VOLATILES THROUGH TIME

John W. Delano, New York Center for Studies on the Origin of Life (NSCORT-NY), Department of Earth and Atmospheric Sciences, University at Albany (SUNY), Albany, NY 12222

The composition (i.e., oxidation state) of the Earth's atmosphere has been a key parameter in some scenarios for the origin of life. If the Earth's atmosphere/hydrosphere system began principally by mantle degassing, the maintenance of a low oxidation state in the atmosphere (at least until life became sustainable against impact sterilization at ~4.2-3.8 Ga; Sleep et al., 1989) depends on the following having occurred: persistence of a low oxidation state in Earth's mantle reservoirs to resupply the atmosphere with reduced mantle-derived volatiles through the interval of 4.2-3.8 Ga at a rate sufficient to compensate for degradation of chemically reduced compounds by solar UV radiation.

Experiments show that Cr abundances in basaltic melts under conditions of spinel-saturation are a function of temperature, melt composition, and oxygen fugacity (e.g., Hanson and Jones, 1998). Since the original abundances of Cr in ancient, mantle-derived magmas can be preserved, measured abundances have been used in the combination with experimental calibration of the Cr oxygen barometer to estimate the oxidation state of the Earth's mantle, and hence the composition of degassed volatiles, through time (back to 3.8 Ga). Results indicate that the mantle reservoirs responsible for generating the greatest volumes of magmas (i.e., basalts) have been at-or-near current oxidation states (i.e., oxidizing) for at least 3.8 Ga. Although other mantle reservoirs appear to have lower oxidation states (e.g., Kasting et al., 1993), these reservoirs may be minor in size and are not currently known to have ever been associated with large degassing events relevant to atmospheric composition.

Hanson, B.Z. and Jones, J.H.: 1998, American Mineralogist, **83**, 669.
Kasting, J.F., Eggler, D.H., and Raeburn, S.P.: 1993, J. Geol., **101**, 245.
Sleep, N.H., Zahnle, K.J., Kasting, J.F., and Morowitz, H.J.: 1989, Nature, **342**, 139.

cA1.5

EXPERIMENTAL INVESTIGATIONS INTO DYNAMIC ORGANIC REACTION NETWORKS AT HIGH T AND P IN AQUEOUS MEDIA

George D. Cody, Nabil Boctor , Jennifer Blank, Jay Brandes, Nabil Boctor, Tim Filley, Robert Hazen, and Hatten Yoder, Jr. Geophysical Laboratory, Carnegie Institution of Washington, 5251 Broad Branch Rd., NW. Washington, DC 20015

It has been argued that life evolved first as a purely metabolic entity and then developed its characteristic replicative properties. It has been further argued that the metabolic biochemistry ubiquitous to life, the tricarboxylic acid (TCA) cycle, was adopted by the earliest emerging living system from its environment rather than being derived through some evolutionary process. This later statement requires that within whatever environment life first emerged, abiotic organic chemistry similar to the reductive TCA cycle must have been intrinsic; i.e. a necessary consequence of the particular set of physical and chemical characteristics of the environment. Clearly, life is an emergent property of a complex dynamic chemical system.

Recently, interest has focused on deep sea hydrothermal vents as potentially promising environments for the development of pre-biotic dynamic chemical systems. A particularly compelling aspect of this hypothesis is that it greatly expands the possibility of life beyond the narrow band of solar luminosity that supports surface liquid water and, therefore, accommodates the possibility of biochemistry similar to that of terrestrial life, emerging within other planetary bodies in our and other solar systems.

We have begun to explore the organic reactions under conditions that mimic aspects of hydrothermal vent systems, i.e. T = 100-300°C, P = 200-2000 Bar, variable fluid composition (eH and pH), and including transition metal sulfide minerals to promote of organic reactions that mimic key metabolic steps. Focusing initially on the system C-H-O we find a well connected chemical network involving a range of reversible reactions defining metastable equilibria. These include Aldo-retro Aldol, hydrogenation- de-hydrogenation, hydration - dehydration, and carboxylation - decarboxylation reactions. The reaction network is sensitive to pressure, temperature, and fluid composition. Mineral catalysts, specifically transition metal sulfides, are crucial for the establishment of a number of the metastable equilibria. A number of routes towards large molecule formation via polymerizations are identified, yielding poly [Aldol], poly [ester], and poly [olefin] type oligomers.

cA1.6

LIPID FORMATION BY AQUEOUS FISCHER-TROPSCH-TYPE SYNTHESIS OVER A TEMPERATURE RANGE OF 100 TO 400°C.

Ahmed I. Rushdi and Bernd R.T. Simoneit
Environmental and Petroleum Geochemistry Group, College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR, 97331, U.S.A.

The formation of lipid compounds during an aqueous Fischer-Tropsch-type reaction was studied with solutions of oxalic acid as the carbon source. The reactions were conducted in stainless steel vessels by heating the oxalic acid solution at discrete temperatures from 100 to 400°C, at intervals of 50°C for two days each. The reaction products were extracted with methylene chloride/methanol (3:1 v/v) and the extracts were concentrated under nitrogen blow down at ambient temperature prior to GC and GC-MS analyses. At a temperature of 100°C only a trace amount of lipids was detected with the predominant products of *n*-alkanes ranging from C_{13} to C_{33}, *n*-alkanols from C_{10} to C_{18}, n- alkanoic acids from C_{14} to C_{18} and phenols. At temperatures above 150°C the lipid components ranged from C_{12} to > C_{33} and included *n*-alkanols, *n*-alkanoic acids, *n*-alkanes, *n*-alkenes and *n*-alkanones, all with no carbon number preference > C_{15}. The *n*-alkanes increased in concentration over the oxygenated compounds at temperatures of 350°C and above, with a slight reduction in their carbon number ranges due to cracking. It was also noted that the *n*-alkanoic acids increased while *n*-alkanols decreased with increasing temperature. At 400°C significant cracking was observed and polynuclear aromatic hydrocarbons and their alkylated homologs, including naphthalene, phenanthrene, flourene, flouranthene, pyrene, chrysene, benzanthracene and benzoflouranthenes, were significant components. At temperatures above 300°C synthesis competes with cracking and reforming reactions. The maximum lipid yield, especially for oxygenated compounds, is in the window of 150-250°C.

cA1.7

ORIGIN OF LIFE WITHOUT BIOPOLYMERS: A LIPID WORLD SCENARIO

Doron Lancet[1], Dafna Ben-Eli[1], David Deamer[2] and Daniel Segré[1]
[1] Dept of Molecular Genetics and the Genome Center, the Weizmann Institute of Science, Rehovot 76100, Israel and [2]Dept. of Chemistry, University of California, Santa Cruz, California 95064, USA

The molecular systems out of which life originally evolved were subject to relatively simple physical and chemical laws. Such primitive prebiotic systems were not endowed with the sophisticated information-coding mechanisms or accurate self-replication capacity that define cellular life today. The exact chemical embodiment of these early systems is uncertain, as are the transitions that led them to a more elaborate biopolymer-based chemistry such as the "RNA World". Here we critically analyze the potential prebiotic role of intermediate-size lipid-like amphiphilic substances, capable of forming non-covalent assemblies, including micelles and bilayers. The continuity of bilayer membranes with similar structures in contemporary cellular life, and the requirement for microenvironments in which high concentrations can be achieved, support the idea that lipid structures were involved in the emergence of molecular systems displaying basic properties of the living state (Deamer, 1997).

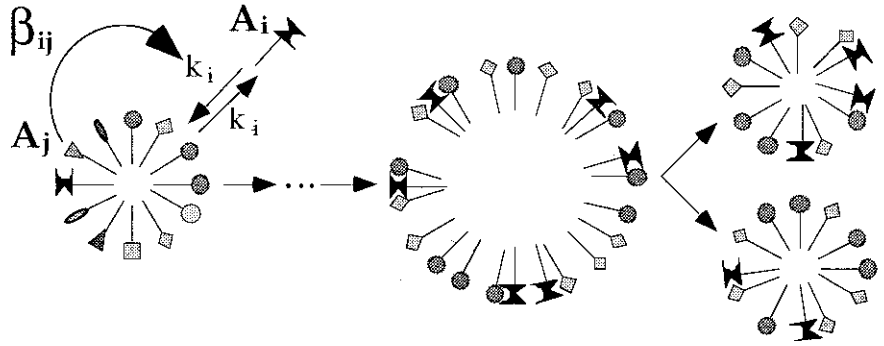


Figure 1. Growing and splitting of heterogeneous micelles with catalytic joining. Amphiphiles are represented by hydrophobic tails (sticks) with diverse hydrophilic heads (shapes). In the absence of catalysis, the molecular species A_i are assumed to join/leave a micelle by a reversible reaction with rate constants k_i and k_{-i}. Mutual catalysis is exerted on joining/leaving of A_i by another molecule A_j (round arrow), with a catalytic enhancement β_{ij}. The composition of the growing assemblies becomes biased due to the occurrence of closed mutually catalytic networks, whereby a few species are present in large counts. When a micelle reaches a critical size, random splitting due to physical forces generates daughter assemblies, whose compositions resemble each other, as well as that of the parent micelle.

cA1.7 continued

The availability of such amphiphilic compounds on early Earth, both from endogenous and exogenous sources, has been amply demonstrated (Deamer, 1997).

We propose a "Lipid World" scenario as an early step in molecular evolution. This concept combines standard lipid chemistry and physics, including basic equations of chemical kinetics and thermodynamics, with the notion of mutually catalytic sets. It may be viewed as a generalization of Luisi's paradigm of self-reproducing lipid structures (Bachman et al., 1992). A probabilistic formalism similar to that involved in combinatorial chemistry is used to describe the interactions within random collections of prebiotic lipid-like molecules, as suggested also by a statistical reexamination of experimental values for membrane mimetic compounds (Fendler, 1982). The model is analyzed through computer simulations with stochastic chemical kinetics rules, based on the Graded Autocatalysis Replication Domain (GARD) equations (Segré et al., 1998). This results in homeostatic preservation of the molecular composition and assembly growth (Figure 1; Segré and Lancet, 1998). With one additional assumption, whereby an amphiphilic assembly may split and generate two distinct new aggregates, a "Morowitz Boundary" concept (Morowitz, 1967; Segré, 1999) can be used to describe the propagation of compositional information to daughter micelles. In other words, in a Lipid World information is stored and propagated as a "compositional genome", rather than in the sequences of biopolymers.

We show that when aggregates of amphiphiles are kept far from thermodynamic equilibrium, a rudimentary form of natural selection can begin, in which more efficient mutually catalytic assemblies gradually prevail. We are now investigating chemically-defined steps in which amphiphilic assemblies, capable of rudimentary evolution, may acquire new capacities, including the emergence of alphabet-based biopolymers (Segré et al., 1999).

Bachman, P.A., Luisi, P.L., Lang, J.: 1992, Nature, 357, 57-59.
Deamer, D.W.: 1997, Microbiol. and Mol.Biol. Reviews, **61**, 239-261.
Fendler, J.H.: 1982, *Membrane Mimetic Chemistry*, Wiley.
Morowitz, H.J.: 1967, Progr. in Theor. Biol., Academic Press, **1**, 35-58.
Segré, D., Lancet, D., Kedem, O. and Pilpel, Y.: 1998, Orig. Life Evol. Biosphere, **28**, 501-514.
Segré, D. and Lancet, D.: 1998, in *Exobiology*, J. Chela-Flores and F. Raulin (eds.), Kluwer, 123-131.
Segré, D., Lancet, D.: 1999, Chemtracts - Biochem. and Mol. Biol., 12(6).
Segré, D., Ben-Eli, D. and Lancet, D. ISSOL 1999.

cA1.8

LAYERED DOUBLE HYDROXIDES AND THE ORIGINS OF LIFE

Joseph W. Boclair, Paul S. Braterman, Brian D. Brister, Jianping Jiang, Shaowei Lou, Zhiming Wang and Faith Yarberry, Department of Chemistry, University of North Texas, Denton, TX 76203, USA

Layered double hydroxides (LDH) are anion-exchanging materials of type $M(III)M(II)_x(OH)_{(2x+2)}Y$ that occur abundantly in nature, and can concentrate, protect, and activate simple organic anionic species of possible relevance to the earliest organisms. For example, they are known (Pitsch *et al.*) to catalyze, and to change the stereoselectivity of, the trimerization of glycolaldehyde phosphate to hexose. We now wish to report progress in the following areas:

1) Internal vs. external uptake of anions. Ferrocyanide does not displace carbonate from synthetic hydrotalcite (Mg:Al LDH carbonate) but is taken up on the outside of the particles only. In other cases, anion uptake is controlled by specific hydrogen bonding requirements rather than by charge density alone, a feature that can be used to control whether uptake will be both internal and external, or external only. These two findings taken together have important implications for specific catalysis by LDH, since specific hydrogen bonding will affect the individual and relative conformations of substrate anions, and anions occupying space in the interlayer will be under tighter constraints than those adsorbed externally.

2) Specific reactions catalyzed by LDH. We have found that the LDH $Mg_2Al(OH)_6Cl$ catalyzes a novel polymerization of cyanide, to give among other products, in a one-pot reaction, a purple-pink material that adheres to the LDH. We are investigating whether this reaction also occurs with hydrotalcite itself, what is the minimum effective concentration of cyanide, and what can be learned about the products.

3) Autocatalysis in LDH formation. We have found that the formation of Cr(III)Ni(II) LDH by hydrolysis of urea is autocatalytic. While we are not seriously suggesting that this exact process played a role in the origins of life, we nonetheless feel that *any* autocatalytic cycle of such simplicity, especially when it leads to a highly structured and catalytically active product, deserves closer examination.

Pitsch, S., Eschenmoser A., Gedulin, B., Hui, S. and Arrhenius, G. : 1995, Origins of Life, **25**, 297

cA1.9

POLYESTER FACILITATED CONDENSATION OF α-AMINO ACIDS NEW MODEL FOR ABIOTIC SYNTHESIS OF PEPTIDES

László Sipos
Erdey-Grúz Tibor Technical School of Chemistry
Debrecen, Hungary

Miller's experiment showed that formation of α-amino acids is always accompanied by the formation of α-hydroxy acids like glycolic acid, lactic acid. Heating α-hydroxy acids around 100 °C, even in the presence of ammonia, gives low molecular weight, water insoluble polymers. If α-amino acids are also present, they build in into the polyester chain, so this polymer matrix could serve as "collecting place" for amino acids under prebiotic circumstances on the ancient mainland(s). Because by the evaporation of water other volatile, monofunctional compounds leave the phase too, this material seems to be an ideal starting material for the synthesis of peptides.

In our experiments we tried to detect whether peptide formation is possible in this polymer matrix. Different kinds of α-amino acid - lactic acid mixtures were heated at 90 °C in an open system. After their hydrolysis with water and subsequent reaction with new supply of α-amino acid, peptide bond containing compounds could be spotted in the solution by HPLC. Repeating the drying-hydrolysis cycle, the amount of peptides increased. By mild hydrolysis the lactic acid moiety remains on both the carboxyl and amino group of the amino acid, so it serves not only as activator on the carboxyl group, but as protecting group on the amino group. Due to this protecting feature of lactic acid, the formation of diketopiperazine could be maintained at low level.

cB1.1

CHLOROACETALDEHYDE CYANOHYDRIN: A POTENTIALLY PREBIOTIC ACETYLATING AGENT

Irene Sust, Albert Alabau and Carles Estévez.
Chemistry Department. Institut Universitari de Ciència i Tecnologia.
Alvarez de Castro, 63. 08100 Mollet del Vallès. Barcelona, Spain.
labquim@iuct.com

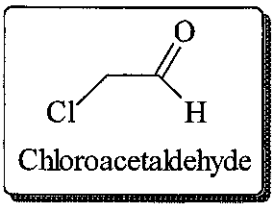
α-Substituted acetaldehydes have been shown to be important prebiotic compounds. For example, cyanoacetaldehyde is a precursor of pyrimidines (Robertson *et al.*, 1996). We suggest that chloroacetaldehyde may have been a prebiotic molecule since it can be produced by the addition of HOCl to acetylene or by direct chlorination of acetaldehyde. Acetylene and acetaldehyde are known to be major prebiotic molecules. Cl_2 has been suggested to play an important role in the early chemical evolution on Earth (Estévez, 1996). The recent discovery of rapid Cl_2 formation when sea-salt particles above its deliquescence point are irradiated with UV light in the presence of O_3 (Oum *et al.*, 1998) indicates a global source of Cl_2 on the present Earth. The mechanism involves the photochemical generation of H_2O_2 from O_3. Photodissociation of H_2O_2 produces two OH radicals which react with chloride ion in the aqueous phase to form Cl. Recombination of two chlorine atoms yields Cl_2. This finding strongly supports the hypothesis of a significant prebiotic source of Cl_2 in the marine boundary layer and in coastal areas since these oxidants were probably formed by water photolysis in the primitive atmosphere of the Earth.

Our primary goal was to investigate the reaction of chloroacetaldehyde with aqueous CN^- solutions. It is known that α-chloroacetaldehyde is converted to acetic acid by the action of cyanide ion. The proposed mechanism involves a rapid cyanohydrin formation, dehydrochlorination to yield acetyl cyanide and rapid hydrolysis of acetyl cyanide to acetic acid (Nowak, 1963). Since acetyl cyanide is a powerful acetylating agent, it is expected to react with nucleophiles other than H_2O to produce acetyl derivatives.

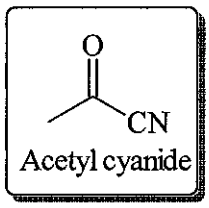
We have been able to synthesize acetanilide with yields as high as 25% from aniline (10^{-2} M), chloroacetaldehyde (3.1×10^{-4} M), and KCN (4.4×10^{-3} M) at 25°C. The maximum yield of acetanilide is proportional to the aniline concentration. The yields are lower at higher pH due to

hydrolysis of acetyl cyanide by OH^-. The initial rate of reaction is proportional to the KCN concentration and is independent of the aniline concentration. The pH-rate profile shows a maximum at pH 9.8 ($t_{1/2}$ = 5 hours). Our kinetic data is consistent with the proposed mechanism, with dehydrochlorination of chloroacetaldehyde cyanohydrin being the rate determining step.

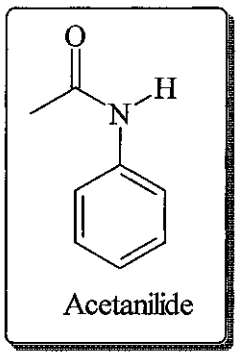
The reaction of chloroacetaldehyde/cyanide mixtures with other prebiotic nucleophiles such as phosphate, thiols, nucleobases, and aminoacids can produce acetylphosphate, thioesters, modified bases such as N^4-acetylcytosine, and *N*-acetylaminoacids. We will present our progress in the synthesis of these important prebiotic compounds.



Chloroacetaldehyde



Acetyl cyanide



Acetanilide

Estévez, C. (1996) Abstracts of the ISSOL'96. P21, page 70.
Nowak, R. M. (1963) J. Org. Chem. 28, 1182-1187.
Oum, K. W., Lakin, M. J., DeHaan, D. O., Brauers, T., Finlayson-Pitts, B. J. (1998) Science, **279**, 74-77.
Robertson, M. P., Levy, M., Miller, S. L. (1996) J. Mol. Evol. **43**, 543-550.

cB1.2

FORMATION OF GLYCOLALDEHYDE PHOSPHATE FROM GLYCOLALDEHYDE IN AQUEOUS SOLUTION

Ramanarayanan Krishnamurthy[1], Gustaf Arrhenius[2], and Albert Eschenmoser[1,3]

[1]The Skaggs Institute for Chemical Biology at The Scripps Research Institute, La Jolla, CA, USA 92037; [2]The Scripps Institute of Oceanography, UCSD, La Jolla, CA, USA 92037; [3]Laboratorium für Organische Chemie, Eidgenössiche Technische Hochschüle, Zürich, Switzerland, CH 8092.

Formation of glycolaldehyde phosphate (GAP), a key ingredient for the formation of sugar phosphates (e.g. ribose-2,4-diphosphate; Müller et al., 1990; Pitsch et al., 1995; Krishnamurthy et al., 1999), under potentially natural conditions is demonstrated by phosphorylating glycolaldehyde with amidotriphosphate (AmTP) in dilute aqueous solutions (as low as 30 μM in glycolaldehyde and 60 μM in AmTP) under near neutral conditions. The phosphorylating reagent AmTP is formed by the reaction of trimetaphosphate (TMP) with aqueous ammonia (Feldmann et al., 1964). In sharp contrast, all attempts to achieve a phosphorylation of glycolaldehyde with trimetaphosphate (TMP) as phosphorylating reagent were unsuccessful.

In this presentation we describe these findings, discuss the mechanism of GAP formation with this reagent (AmTP) and point to the potential of amidotriphosphate as a substrate-specific phosphorylation reagent.

Feldmann, V. W. and Thilo, E. Z.: 1964, Z. Anorg. Chem. **328**, 113-216.

Krishnamurthy, R., Pitsch, S., Arrhenius, G.: 1999, Origins Life Evol. Biosphere (in press).

Müller, D., Pitsch, S., Kittaka, A., Wagner, E., Wintner, C.E., Eschenmoser, A.: 1990, Helv. Chim. Acta **73**, 1410-1468.

Pitsch, S., Eschenmoser, A., Gedulin, B., Hui, S., Arrhenius, G.: 1995, Origins Life Evol. Biosphere **25**, 294-334.

cB1.3

Novel Phosphoxy-derivatives of Cytosine, Uracil and Thymine under Far UV Irradiation

Li Hongxia, Wang Wenqing* and Wu Jilan
Technical Physics Department,Peking University,Beijing100871,China

Three novel photoproducts 6-phosphoxy-cytosine (uracil and thymine) have been found in the photolysis of nucleobase phosphate solution under far UV irradiation. The photolysis of bases was enhanced sharply in the presence of phosphate. The photoproducts were isolated and purified by anion exchange column of Amberlite CG 400 II, 200-400 mesh. The resin was converted to formate form, utilizing formate form giving better pH control, more available anion and a lower specific replacement power than Cl form..

6-phosphoxycytosine ($C_4H_6N_3O_5P$) was identified by use of ^1H, ^{31}P-NMR spectroscopy, element analysis, ultraviolet, infrared spectroscopy and electron impact mass spectroscopy. The photoproducts of uracil ($C_4H_5N_2O_6P$) and thymine ($C_5H_7N_2O_6P$) were isolated and identified as 6-phosphoxy-uracil (thymine) by UV, ^1H-NMR and LC/MS/MS. These phosphoxy compounds are novel, irreversible and stable contrary to the photohydrates, and may have important roles in the inactivation and mutation of living organisms and viruses by ultraviolet light which caused irreversible DNA damage.

According to the structures of the photoproducts and the kinetics of the photolysis reaction, a mechanism is supposed as following: the phosphate dianion absorbed far UV light to transit electronically excited state, then formed $HPO_4^{\cdot-}$ radical and released an electron to the solvent. Oxygen scavenges the aqueous electron which motivates the formation of phosphate radical. Then phosphate radical attack C-6 of nucleobase to form 6-phosphoxycytosine (uracil and thymine).These types of compounds have not been obtained since Beherns (1988) found the radical of phosphoxy-cytosine with ESR spectroscopy.

Phosphate can enhance not only the photolysis of nucleobases, but also the photolysis of nucleosides and nucleotides. This phosphate effect seems to produce serious handicap of life origin from RNA world. However, it was found that it can be inhibited by amino acids. Furthermore, the inhibition effect of L-, D-leucine on the photolysis of 5'-CMP(Pan,X.M.,et al ,1995) in phosphate solution showed obvious chirality difference which may be

cB1.3 continued

D- sugar constitued in 5'-CMP. Amino acids acted as photoprotectants and antioxidants is one useful apporach to inhibit the effect of UV-induced damage to particular cellular constituents. Nucleobases and amino acids are complementary each other in the chemical evolution of primordial Earth.

We found that natural product flavonoids which were reported to be effective ·OH and $O_2^{\cdot-}$ radicals scavengers (R. Lois, 1994) can inhibit the photolysis of nucleobases. The mechanism is supposed that flavonoids—quercetin and rutin compete phosphate radical with nucleobase. It was found that the protective role of quercetin is more effective than that of rutin. These protective effect is important which shed light on avoiding DNA damage induced by UV irradiation.

The significance of this work is that we first isolated and identified three novel compounds--6-phosphoxybases which caused irreversible DNA damage. Second, the photolysis of nucleobases is inhibited by amino acids which demonstrates that the fundamental constitutes---amino acids and nucleobases are complementary each other in the chemical evolution. We also found natural product flavonoids which widespread in nature can inhibit this photolysis.

Cader, J.: 1985, Can. J. Chem. **63**, 2861.
Gehrens, G. : 1988, J.Chem. Soc. Perkin Trans, 305.
Paul W.Doetsch: 1995, Biochemistry, **34**, 737.
Pan, X.M., Wang W.Q.: 1995, J. Biol. Phys., **21,** 61.
Lois, L.: 1994, Planta, **194**, 498.
Steenken, S. : 1983, J. Amer. Chem.Soc., **105**,4380
Yamagata,Y.: 1991, Nature, **352** (8), 516.

cB1.4

PREBIOTIC FORMATION OF ADP AND ATP FROM AMP, CALCIUM PHOSPHATES AND CYANATE IN AQUEOUS SOLUTION

Yukio Yamagata

Laboratory of Chemical Evolution
1408 Dynasty-Asanogawa, 15-60 Showeimachi, Kanazawa
Ishikawa 920-0846, Japan

Adenosine-5'-triphosphate was synthesized by the phosphorylation of adenosine-5'-diphosphate in aqueous solution containing cyanate as a condensing agent and insoluble calcium phosphate produced from phosphate and calcium chloride. In a similar manner, adenosine-5'-diphosphate was synthesized from adenosine-5'-monophosphate. When the experiment was carried out in the conditions of 4°C and pH 5.75, the formation of adenosine-5'-diphosphate and adenosine-5'-triphosphate from adenosine-5'-monophosphate was observed in the yields of 19% and 7% after 22 days, respectively. The yields were strongly dependent on the pH and a pH of 5.5-6.0 was the most effective. The other nucleoside-5'-triphosphates were also produced from their respective diphosphates.

Parallel experiments used cyanamide and dicyandiamide as the condensing agent did not produce any phosphorylated products.

cB1.5

PREBIOTIC SYNTHESIS OF NUCLEOTIDES

Geoffrey L. Zubay
Fairchild Center, Columbia University, NYC, NY 10027

If one accepts the concept of an RNA-only world, one must deal with the question of how the first nucleotides were made. This question was extensively investigated in the 1960s and the early 1970s by others (Joyce, 1989). Much progress was made but many problems were left unresolved. For the past six years we have been exploring possible prebiotic reactions with the goal of finding a feasible overall pathway for the synthesis of the nucleotides that were incorporated into the first RNA molecules. For reasons that will be discussed, our studies have focused on the synthesis of the two purine nucleotides, adenylic acid and inosinic acid. In this report, I describe three areas in which some progress has been made.

Starting with the problem of purine synthesis, previous studies showed that aminoimidazole carboxamide (AICA) and aminoimidazole carbonitrile (AICN) were converted in low yields into hypoxanthine and adenine respectively after prolonged heating in sealed tubes containing an aqueous solution of HCN (Joyce, 1989). In more recent investigations we have observed that AICA and AICN were efficiently converted into these two purines by incubating them at 75 C in open wells containing an aqueous solution of ammonium formate (Zubay, 1994). As the reaction progressed the water evaporated and finally the unreacted ammonium formate sublimed leaving a residue in which the purine product predominated. It is noteworthy that the reaction between AICA and ammonium formate bears a striking resemblance to the parallel reaction in the de novo biochemical pathway for inosine monophosphate.

On the subject of ribose synthesis little progress had been made in the synthesis of neutral ribose since it was discovered that small amounts of ribose are formed when an aqueous solution of formaldehyde is heated in the presence of calcium hydroxide. We have found that under milder conditions, lead (PbII) is an excellent catalyst for ribose synthesis (Zubay, 1998). Synthesis of the other straight chain aldopentoses is also stimulated in the presence of Pb(II). It is not clear if they are formed de novo or from ribose.

cB1.5 continued

PREBIOTIC SYNTHESIS OF NUCLEOTIDES

The unique effectiveness of Pb(II) is believed to be due in part to the exceptionally low pKa of this metal ion (pKa 7.7).

Finally, improved conditions for the synthesis of 5'-NMPs from nucleosides and of tri-and tetraphosphate derivatives from 5' –NMPs have also been found and will be discussed (Reimann and Zubay, in press).

Despite the gains that have been made there are many problems that remain unsolved. The linkage of ribose to the purine base is one of the biggest and this is where we are concentrating our effort at the present time.

Joyce, G.F.: 1989, *Nature* **338**, 217.
Reimann, R. and Zubay, G: 1999, *Origins of Life*, in press.
Zubay, G: 1994, *Chemtracts-Biochem and Mol Bio* 5, **179**.
Zubay, G: 1998, *Origins of Life*, **28**, 1998.

cB1.6

KINETIC EVIDENCE FOR WEAK COOPERATIVITY IN THE SELF-ASSOCIATION OF PYRIMIDINE NUCLEOTIDE DERIVATIVES IN AQUEOUS SOLUTIONS AND IN THE PRESENCE OF DIVALENT METAL IONS.

Kanavarioti, Anastassia and Lee, F. Lynn
Department of Chemistry and Biochemistry, University of California
Santa Cruz, CA 95064, USA

Stacking of nucleotides in water provides the major force for self-asscociation as shown by H^1 NMR upfield shifts of the ring protons, decreasing molar osmotic coefficients and hypochromic effects in the UV as a function of increasing concentration of the nucleotide. The relatively larger area of the purine bases compared to the pyrimidine bases leads to stronger complexation by stacking in the order purine•purine > purine•pyrimidine > pyrimidine•pyrimidine. Available data using different methods fit best an isodesmic model where the association constant of one molecule, such as cytidine (C), to another, K_1, is equal to the association constant, K_{n-1}, of the n-th cytidine monomer to a stack of n-1 cytidine units where $n \geq 2$ (Martin, 1996). It is only with guanosine derivatives where self-association is shown to fit a cooperative model where $K_1 < K_2 = K_n$.

$$C + C \overset{K_1}{\Leftrightarrow} C_2, \quad C_2 + C \overset{K_2}{\Leftrightarrow} C_3, \quad C_3 + C \overset{K_3}{\Leftrightarrow} C_4, \ldots\ldots \quad C_{n-1} + C \overset{K_{n-1}}{\Leftrightarrow} C_n$$

In exporing the oligomerization of phosphoimidazolide activated nucleotides, pN*, in water at 23° C, pH 7.5 and in the presence of metal ions (Mg^{2+} and Mn^{2+}, Kanavarioti, 1997) we observed that the percent yield of dimers and short oligomers increases as a function of initial monomer concentration up to 0.3 to 0.4 M. However, at concentration of monomer higher than 0.4 M the product distribution becomes practically independent of substrate concentration even at early reaction times.

The observation of first-order kinetics for product formation can be rationalized by self-association that results in practically complete incorporation of the substrate in complexes.

We have used the yield of condensation product as a measure of the fraction of the substrate being incorporated in complexes or stacks. Analysis of product yield as a function of substrate concentration allows calculation of self-association constants for adenosine, cytidine and uridine derivatives. Computer simulations were performed using Microsoft Excell on a PowerMac and three basic models were tested: The isodesmic model with $K_1=K_2=K_{n-1}$, a model of self-association which leads to "dimers only", i.e. two-unit complexation, with $K_1\neq 0$ and $K_2=K_3=K_{n-1}=0$, and the cooperative model with $K_1<K_2=K_3=K_{n-1}$. Attempts were made with a cooperative model where $K_1<K_2<K_3=K_4=K_{n-1}$ but this did not improve the fit. Under most conditions tested the data with adenosine, cytidine and uridine derivatives fit the coooperative model and allow the estimation of K_1 and K_{n-1}. This conclusion is unprecedented for the above three nucleotides and rather unexpected for the two pyrimidines. The enhanced self-association and cooperativity observed with *pN, compared to the parent compounds, is attributed to an enhanced affinity for stacking provided by the imidazole moiety and the metal ion.

Kanavarioti, A.: 1997, Origins Life Evol. Biosphere **27**, 357-376.
Martin, R. B.: 1996, Chem. Rev. 96, 3043-3064.

cB1.7

PREBIOTIC SYNTHESIS: OLIGOMERIZATION OF MONOMERS IN THE VICINITY OF SUBMARINE HYDROTHERMAL VENTS

Koichiro Matsuno
Dept. BioEngn, Nagaoka Univ. Technol., Nagaoka 940-2188, Japan

Prebiotic oligomerization of monomers such as amino acids requires an organization of transforming preceding products constantly into succeeding reactants while supplying a sufficient amount of energy to drive the synthetic reactions. A likely candidate for such a primitive organization helping prebiotic monomers oligomerize in a stepwise manner among themselves could be submarine hydrothermal vents in the Archaean ocean. We have constructed a flow reactor simulating a submarine hydrothermal system. A main feature of our flow reactor is to let the high-pressure high-temperature water (24MPa, 200°-250°C) carrying reactants enter into the high-pressure low-temperature water (24MPa, 0°C) and to repeatedly circulate the fluid in the closed path consisting of both the hot and cold regions. Main results we have obtained so far are summarized below.

For the reaction solution of glycine dissolved into pure water with no pH control, no condensing agents and no added salts, we observed:

(1) the oligomerization of glycine proceeded in a stepwise manner from shorter to longer oligoglycine in time,
(2) the initial growth of shorter oligomers such as diglycine and triglycine were exponential in time,
(3) when the flow rate of the reaction fluid decreased, the oligomerization was enhanced up to hexaglycine.

When copper ions were further added to the glycine solution at the pH of 2.5 adjusted by HCl,

(4) the oligomerization of glycine was further enhanced up to octaglycine.

The basic operation of our flow reactor was cyclic and stationary in the sense that the preceding products was constantly converted into the further reactants. Nonetheless, the products themselves exhibited a temporally unidirectional character of changes in that the extent of oligomerization was enhanced in time. A likely mechanism that could explain some aspects of the present results could be that as the reaction fluid circulated the closed path of the reactor repeatedly, the oligomerization may be enhanced until the two processes could equilibrate with each other if ever possible.

cB1.8

THE SEMI-ENZYMATIC ORIGIN OF THE BIOSYNTHETIC PATHWAYS

H. James Cleaves and Stanley L. Miller
Department of Chemistry and Biochemistry
University of California, San Diego
La Jolla, CA 92093-0506

There have been several proposals for the origin of the metabolic pathways involving the stepwise development of the pathways in either the backward (Horowitz, 1945) or forward (Granick, 1957) directions by recruitment of new enzyme function. The patchwork hypothesis (Jensen, 1976) involves the recruitment of non-specific enzymes in any order. The semi-enzymatic theory for the origin of the metabolic pathways (Lazcano and Miller, 1999) holds that the earliest pathways were partially or wholly non-enzymatic. While it is clear that certain metabolic pathways must include some enzymatic reactions, there appear to be several which proceed completely non-enzymatically, although in low yield.

The central importance of the coenzymes in metabolic processes suggests that their biosynthetic pathways should have been laid down very early in the evolution of metabolism. We find that many of the coenzymes can be produced in substantial yields under plausible early biological conditions (moderate temperature, neutral pH and low concentrations) via reaction mechanisms which closely mimic the biological pathways. Our results suggest that many of the biological pathways began non-enzymatically or semi-enzymatically and were taken over as the full host of required enzymes was developed from gene duplication.

As an example, we find that the non-enzymatic reaction of dihydroxyacetone phosphate (DHAP) and aspartic acid proceeds in a one-pot reaction all of the way to quinolinic and nicotinic acids. Quinolinic acid is the precursor in all pyridine nucleotide biosyntheses. DHAP would presumably have been present in an organism which was able to biosynthesize ribose, and aspartic acid would have been available to a primitive heterotroph.

Preliminary studies demonstrate that several other coenzymes and amino acids are attainable under similar conditions. The facile synthesis of such compounds suggests that many of the coenzymes present in modern biochemistry are inevitable by-products of a biochemistry based on amino

cB1.8 continued

acids and sugar phosphates. Thus, these arose in biochemistry in the DNA/Protein World or even as early as the RNA World.

Granick, S. :1957, Ann. NY. Acad. Sci. 69, 292-308
Horowitz, N. :1945, Proc. Nat. Acad. Sci. USA 31, 153-157
Jensen, R.A. :1976, Ann. Rev. Microbiol. 30, 409-425
Lazcano, A. and Miller, S.L. :1999, J. Mol. Evol. (in press)

cB1.9

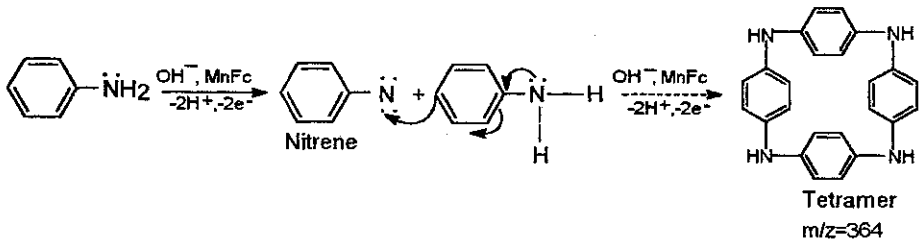
ROLE OF METAL FERROCYANIDES IN CHEMICAL EVOLUTION

Interaction of Manganese Ferrocyanide with Aromatic Amines and Its Relevance in Chemical Evolution

Tanveer Alam and Kamaluddin
Chemistry Department, University of Roorkee, Roorkee - 247 667, India

It is believed that chemical system leading to the origin of life evolved on clay and mineral surfaces. Insoluble metal ferrocyanides which form a special class of inorganic materials due to ease in formations and for the ion exchange properties (Kourim *et al.*, 1964). These metal ferrocyanides are also considered to be the active surface catalysts for many prebiotic reactions (Kamaluddin *et al.*, 1990, 1994; Viladkar *et al.*, 1994,1996). The present investigations describes the oxidation of aromatic amines in presence of manganese ferrocyanide.

Manganese ferrocyanide (MnFc) was prepared by following the method reported by Kourim *et al.* (1964). For the oxidation of aniline, *p*-toluidine and *p*-chloroaniline on MnFc following procedure was adopted. Buffered amine solution (10 ml, 1.0×10^{-3} M) was added to 100 mg of MnFc and suspension was shaken to 1 h and then allowed to equilibrate at 25°C for 48 hrs. Reddish brown coloured products were deposited on the surface of MnFc. These coloured products were extracted with benzene. The coloured products soluble in benzene were separated and concentrated for GC-MS and IR studies. The final oxidation products of aniline (Scheme 1), *p*-toluidine (Scheme 2) and *p*-chloroaniline (Scheme 3) are presented here.



Scheme 1

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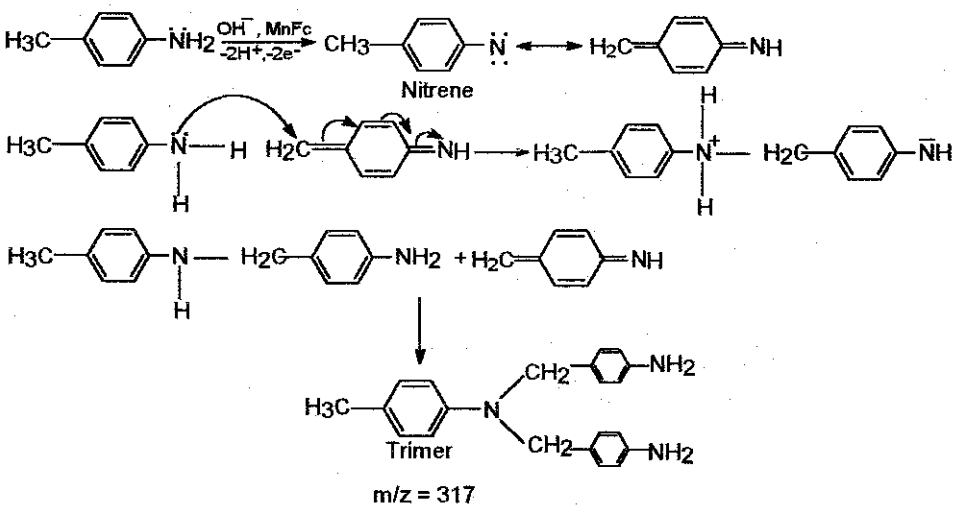
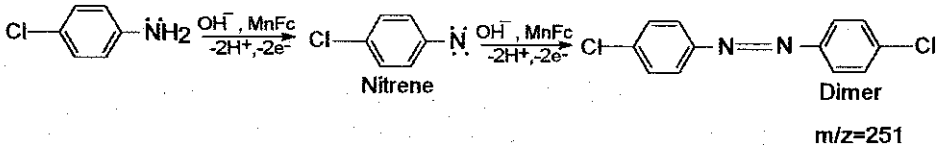
ROLE OF METAL FERROCYANIDES IN CHEMICAL EVOLUTION



Scheme 2



Scheme 3

It is important to note that these reactions occurred only in alkaline medium at pH 7-9. Manganese which is in its oxidation state of II in $Mn_2[Fe(CN)_6]$ possibly changes to its stable zero oxidation state in aqueous medium (Lee, 1991).

Kamaluddin, Nath, M., Deopujari, S. W. and Sharma, A. : 1990, Orig. Life. Evol. Biosphere, **20**, 259.

Kamaluddin, Nath, M. and Sharma, A. : 1994, Orig. Life Evol. Biosphere, **24**, 469.

Kourim, V., Raise, J. and Million, B. : 1964, J. Inorg. Nucl. Chem., **26**, 1111.

Lee, J.D. : 1991, *Concise Inorganic Chemistry*, Chapman and Hall, London, p. 734.

Viladkar, S., Alam, T. and Kamaluddin : 1994, J. Inorg. Biochem., **53**, 69.

Viladkar, S., Rachna, A. and Kamaluddin : 1996, Bull. Chem. Soc. Jpn., **69**, 95.

i2.1

CHIRALITY OF AMINO ACIDS IN THE MURCHISON METEORITE--A HISTORICAL PERSPECTIVE

Keith A. Kvenvolden
USGS, 345 Middlefield Road, MS999, Menlo Park, CA 94025, USA

The fall of the Murchison meteorite in southeastern Australia in 1969 changed the course of studies in organic cosmochemistry. Samples from this meteorite provided material for the first unambiguous identification in 1970 of extraterrestrial amino acids (Kvenvolden et al., 1971). The distribution of optical isomers (enantiomers) of five protein and five non-protein amino acids were determined on an inside sample by gas chromatography. However, accurate measurements of enantiomeric ratios (D/L) of these amino acids were difficult to achieve by the methods available at the time. In the final analyses, four of the chiral protein amino acids appeared to have a slight excess of L-amino acids, but the non-protein chiral amino acids, including isovaline, were racemic. It was concluded that the slight excess was from minor terrestrial contamination and that all of the chiral amino acids were likely present in the meteorite as racemic mixtures.

Using more sensitive techniques, Bada in 1997 (see Kvenvolden et al., submitted) focused on the most abundant amino acids in inside and outside portions of the same sample of Murchison meteorite analyzed previously. The enantiomeric ratios of the protein amino acids in the inside portion of the meteorite appeared to be slightly lower than values determined in 1970, but in agreement with the earlier work within the uncertainty of the measurements. An exception was aspartic acid, the enantiomeric ratio of which had a high degree of uncertainty because of its very low concentration. The only chiral non-protein amino acid determined was isovaline which was racemic, in agreement with the earlier results.

The enantiomeric ratios of aspartic and glutamic acids and alanine in the outside sample were 30 to 60% lower than the ratios in the inside sample, whereas isovaline was racemic. These results indicate that the outside of the meteorite had been exposed to significant terrestrial contamination and serve as a warning that samples of the Murchison meteorite can be affected to varying degrees by terrestrial influences.

Kvenvolden, K.A., Lawless, J.D. and Ponnomperuma, C.:1971, Proc. Nat. Acad. Sci. USA **68**, 486.
Bada, J.L. *in* Kvenvolden , K.A., Glavin, D.P. and Bada, J.L.: submitted, *Perspectives in Amino Acid and Protein Geochemistry*, Ed. by G.A. Goodfriend, Carnegie Institution of Washington.

i2.2

METEORITE AMINO ACIDS AND THE ORIGIN OF HOMOCHIRALITY

John Cronin and Sandra Pizzarello, Department of Chemistry and Biochemistry, Arizona State University, Tempe AZ, USA 85287-1604

Meteorites carry a record of presolar, nebular, and early solar system processes. Interestingly, in carbonaceous chondrites of the CM and CI types, this record includes evidence for an episode of organic chemical evolution during which numerous organic compounds were formed, including an extensive suite of amino acids. These meteoritic compounds are commonly viewed as representative of exogenously delivered organic matter present on the early earth.

Recently, small (1-9%) L-enantiomer excesses have been observed in six α-methyl-α-amino alkanoic acids extracted from the Murchison and Murray CM chondrites. The meteorite extracts were fractionated by ion-exchange and reverse-phase chromatographies and the amino acids analyzed by gas chromatographic-mass spectrometric analysis of their N-trifluoroacetyl or N-pentafluoropropionyl-isopropyl esters on Chirasil-L/D-Val phases. The amino acids with enantiomeric excesses are either unknown or rare in the terrestrial biosphere. Enantiomeric excesses were not observed in four α-H-α-amino alkanoic acids analyzed or were attributed to terrestrial contamination of the meteorites. The substantial excess of L-alanine reported by others was not seen in reverse-phase fractionated extracts of either meteorite.

The enantiomeric excesses reported for the α-methyl amino acids may be the result of partial photoresolution of racemic mixtures as a result of exposure to ultraviolet circularly polarized light in the presolar cloud. It is suggested that the α-methyl-α-amino alkanoic acids may have been significant in the origin of homochirality in view of their resistance to racemization and the possibility of amplification of their enantiomeric excesses suggested by the strong tendency of their polymers to assume chiral secondary structures.

Cronin, J. R. and Pizzarello, S.: 1997, Science, **275**, 951-955.
Pizzarello, S. and Cronin, J. R.: 1998, Nature **394**, 236.

i2.3

PENTOPYRANOSYL NUCLEIC ACIDS

Albert Eschenmoser

The Skaggs Institute for Chemical Biology at The Scripps Research Institute, La Jolla CA, USA 92037 and Laboratorium für Organische Chemie, Eidgenössiche Technische Hochschüle, Zürich, Switzerland, CH 8092.

All four members of the family of pentopyranosyl-(2'→4')-oligonucleotide systems that contain β-ribo-, β-xylo-, α-lyxo- or α-arabinopyranosyl units as repeating sugar building blocks are found to be much stronger Watson-Crick base-pairing systems than RNA. The α-arabinopyranosyl system is the strongest of all, in fact, it belongs to the strongest oligonucleotide base-pairing systems known. We conclude that, whatever the chemical determinants by which nature selected RNA as a genetic system, maximization of base-pairing strengths within the domain of pentose derived oligonucleotide systems was *not* the critical selection criterion.

The lecture discusses selected properties of pentopyranosyl oligonucleotides and reports on most recent results from the Scripps and ETH laboratories.

Beier, M.; Reck, F.; Wagner, T.; Krishnamurthy, R.; Eschenmoser, A.: 1999, Science **283**, 699.

Bolli, M.; Micura, R.; Eschenmoser, A.: 1997, Chemistry & Biology, **4**, 309.

Micura, R.; Kudick, R.; Pitsch, S.; Eschenmoser, A.: 1999, Angew. Chem. **111** (in press).

c2.4

ASTRONOMICAL SOURCES OF CIRCULAR POLARIZATION AND THE ORIGIN OF HOMOCHIRALITY

Jeremy Bailey
Anglo-Australian Observatory, PO Box 296, Epping, NSW 1710, Australia

The detection of an excess of left-handed amino acids in the Murchison and Murray meteorites suggests that the homochirality of biological molecules may reflect an asymmetry already present in the primordial material from which the solar system formed. If this is the case the most likely source of the asymmetry is the action of ultraviolet circularly polarized light (UVCPL) which has been demonstrated to be capable of selecting enantiomers by asymmetric photolysis.

Very few astronomical sources produce circularly polarized light (linear polarization is much more common and much better studied). Synchrotron radiation from magnetic neutron stars has been suggested as a possible source of UVCPL. However, it is difficult to produce significant circular polarization in this way, and none is observed in the best example of such a source, the Crab nebula and its pulsar.

Isolated magnetic white dwarfs and magnetic white dwarf binaries (AM Herculis binaries or polars) can be strongly circularly polarized (up to 50%) but are unlikely to be associated with molecular clouds or star formation regions.

Recent observations at IR wavelengths, however, show that substantial levels of circular polarization are present in reflection nebulae, the polarization probably being the result of scattering from aligned non-spherical dust grains. This mechanism produces polarized light exactly when and where it is required, in regions where star formation is occuring and organic molecules are known to be present. The dust obscuration to these regions means that we are as yet unable to establish observationally whether the polarization extends to shorter wavelengths, but model calculations suggest this is possible.

c2.5

A SOLUTION TO THE PROBLEM OF THE ORIGIN OF BIOCHIRALITY BASED ON OBSERVATIONAL AND EXPERIMENTAL EVIDENCE

Stanley I. Goldberg, Department. of Chemistry, University of New Orleans, New Orleans, LA, USA 70148-2802

The long standing problem of the origin of biochirality (homochirality) has resisted solution because enantiomers possess identical physical and chemical properties, so differences upon which to base possible separations cannot be evoked.

While homochirality is an indispensable factor in contemporary biochemical life, it also appears to have been required for the emergence of life as well for two compelling reasons. First, a homochiral prebiotic world would have neatly avoided the problem of an impossibly large number of configurational isomers resulting from stereochemically random assembly of *D* and *L* monomers into biopolymers such as peptides. Even the formation of a peptide of modest size, say one consisting of only twenty-five amino acid residues, would have meant the stereo-random formation of 2^{25} or 33,554,432 configurationally isomeric peptides; only one of which would have been the all *L*-peptide found in contemporary life. The second reason arises out a number of experimental and theoretical studies all consistent with the view that the presence of both enantiomers, even when one form is in low concentration relative to the other, will prevent or seriously inhibit development of vital biochemical processes (Bonner, 1995, and refs. therein).

Both difficulties are avoided if enantiopure chiral material were present on the primitive Earth, and this paper provides such a solution. It brings together and synthesizes recent observations on the delivery of nonracemic material to Earth (Cronin and Pizzarello, 1997) with older experimental work on phase relationships of enantiomers (Jacques *et al,* 1981) to reveal how global accumulations of enantiopure biologically relevant material could have formed on the early Earth.

Bonner, W. A.: 1995, Origins of Life Evol. Biosphere **25**, 175.
Cronin, J. R.: 1997, Science **275**, 951.
Jacques, J., Collet, A. and Wilen, S. H,: 1981, *Enantiomers, Racemates, and Resolutions*, John Wiley & Sons, New York, especially, Chap. 3.

c2.6

ENANTIOSELECTION THROUGH CHIRAL CONFORMATIONS

Gyula Pályi,[a] Claudia Zucchi,[a] Roland Boese,[b] Miklós Szabó,[c] Robert Szilágyi,[c] and Lajos Bencze[c]

[a] Department of Chemistry, University of Modena, Via Campi 183, I-41100 Modena, Italy

[b] Institute of Inorganic Chemistry, University of Essen, Universitätstrasse 5/7, D-45117 Essen, Germany

[c] Müller Laboratory, Department of Organic Chemistry, University of Veszprém, Egyetem-u.6, H-8200 Veszprém, Hungary

Several apparently symmetric molecules develop chiral conformations along their rotation pathes. The population of these rotamer states depends upon the activation and conformational energies which can result, for example, in the separation of conformational enantiomers by crystallization. Crystallization, from this point of view is a 3D polymerization, driven by the formation of lattice-stabilized supramolecular diastereomers.

We performed an experimental study of this phenomenon: cca 20 alkylcobalt carbonyl tertiary phosphine complexes were designed, prepared and structurally characterized (spectra, X-ray diffraction) for this purpose. The structures of these complexes were systematically built up by complexing first, ligands which were configurationally achiral, but potentially could develop chiral conformers, then, configurational chirality was introduced into the alkyl, then into the phosphine ligand, finally into both types and the evolution of chiral conformations was followed along these changes.

We observed that the alkyl and the phosphine ligands develop chiral conformations, in crystalline phase, in a *concerted* manner. The concertedness of these intramolecular processes lead to the elimination of some statistically possible conformers, resulting in *enantioselection.*

The possible intramolecular pathways conducting to the preparatively and structurally observed selectivities were analyzed by molecular mechanics and MO calculations.

The template-role of the metal in the stabilization of chiral conformations, as well as the relevance of these phenomena to the evolution of biological homochirality will be discussed.

c2.7

CHIRAL AUTOCATALYSIS ON SOLID SURFACES: A SOURCE OF CHIRAL ASYMMETRY

Dilip K. Kondepudi
Department of Chemistry, Wake Forest University,
Winston Salem, NC 27109.

Spontaneous generation of large chiral asymmetry can easily be realized in stirred crystallization of $NaClO_3$ and $NaBrO_3$ (Kondepudi et al. 1990, 1993). The same can also be realized in stirred crystallization of 1,1'-binaphthyl from the melt (Kondepudi et al., 1999). This happens because chirally autocatalytic *secondary nucleation* occurs when a crystallizing solution or melt is stirred during the processes of crystallization. The enantiomeric excess produced in such crystallizations are generally greater than 80%, but which enantiomer will dominate in any particular crystallization is unpredictable -- it is a random process.

Secondary nucleation is a process in which crystal nuclei are generated in the vicinity of an existing crystal. If the crystallizing compound is an achiral molecule crystallizing in chiral forms, the crystallization is chirally autocatalytic, i.e., the secondary nuclei generated in the vicinity of an L-crystals, for example, are predominantly of the L-form. These crystals are then dispersed by the convecting fluid. The same is true for a rapidly racemizing chiral compound (such as 1,1'-binaphthyl close to its melting point) that crystallizes in chiral forms. Since the enantiomeric excess produced is very high, we see that there is a strong chiral selectivity in the vicinity of the surface of a chiral solid.

In the synthesis of a chiral cobalt complex (Asakura et al., 1998) similar chiral selectivity in the vicinity of chiral crystals of the product was observed. With this and the above results, we see that chiral autocatalysis generally occurs in the vicinity of solid chiral surfaces.

Kondepudi, D.K., Kaufmann, R. and Singh, N.: 1990, Science **250**, 975
Kondepudi, D.K., Bullock, K., Digits, J.A., Hall, J.K. and Miller J.: 1993, J. Am. Chem. Soc. **115**, 10211
Kondepudi, D.K., Asakura K., and Laudadio, J.: 1999, J. Am. Chem. Soc. in Press
Asakura, K., Kondepudi, D.K., and Martin, R.:1998, Chirality **10**, 343

P2.1

RANDOM CHIRAL ASYMMETRY GENERATION IN AUTO-CATALYTIC NONEQUILIBRIUM SYSTEMS

Kouichi Asakura*, Akihito Ikumo*, Shuichi Osanai*, Dilip K. Kondepudi**
*Department of Applied Chemistry, Keio University, 3-14-1, Hiyoshi, Yokohama, Japan 223-8522
**Department of Chemistry, Wake Forest University, Winston-Salem NC, USA 27109

Most chemical reactions may be considered essentially homogeneous though there are random fluctuations in all physical variables such as concentrations and temperature. At equilibrium, these fluctuations are insignificant and the system is stable to these extremely small fluctuations. In a nonequilibrium system that has autocatalytic reactions, however, the system may not be stable to fluctuations: a small fluctuation may be amplified. Chirally autocatalytic systems that are far from thermodynamic equilibrium are particularly interesting because they are capable of amplifying small chiral asymmetries that arise randomly. This may result in random generation of large enantiomeric excess (ee) in the product.

We found such random generation of chiral asymmetry in the synthesis of a chiral cobalt complex (Asakura, K. *et al.*). In contrast to chiral asymmetry generation in stirred crystallization (Kondepudi, D. K. *et al.*), the system we studied involves one homogeneous phase and it produces a chiral compound. The ee of the product is found to vary randomly from run to run.

The growth of a fluctuation in a small volume depends on the rate of autocatalytic generation of the compound, which increases the local concentration, and the rate of diffusion, which decreases the concentration. A concentration fluctuation will grow, if it is able to overcome diffusion. Thus, there is a threshold for the growth of a random fluctuations in ee, in a chirally autocatalytic systems. The enantiomeric excess of a resulting chiral product is expected to randomly fluctuate from run to run.

Asakura, K.; Kondepudi, D. K.; Martin, R. : 1998, Chirality **10**, 343.
Kondepudi, D. K.; Kaufman, R.; Singh, N. : 1990, Science **250**, 975.
Kondepudi, D. K.; Laudadio, J.; Asakura, K. : 1999, J. Am. Chem. Soc. To appear.

P2.4

A HYPOTHESIS ON DEVELOPMENT OF HOMOCHIRALITY OF PEPTIDES

Toratane Munegumi* and Akira Shimoyama+
Oyama National College of Technology, Oyama, Tochigi, 323-0806,Japan
+Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Many hypotheses (Bonner, 1991) have been proposed to explain the homochirality of biomolecules with the predominance of one homochiral structure in amino acids or monosaccharides. However, the predominance in the homochiral structure of those monomeric compounds does not always ensure the homochirality in their oligomers and polymers. Some sorts of reasonable scenarios should be also proposed for the formation processes of homochiral oligomers and polymers from the homochiral monomeric compounds. In the previous ISSOL meeting (Munegumi and Shimoyama, 1996), we proposed a new hypothesis on the development of homochirality of peptides. We notified the higher logP values of heterochiral Ala-oligopeptides (P:the partition coefficient between octanol and water) comparing with homochiral Ala-oligopeptides, and postulated the existence of separation processes of these peptides.

In this study, logP values of cyclic dipeptides composed of primitive amino acids (Gly, Ala, Val, and Asp) were calculated by using a software, CAche logP. The calculation results showed that the heterochiral cyclic dipeptides (L-L or D-D) have higher logP values than homochiral ones (L-D or D-L). Therefore, it was found that the cyclic heterochiral dipeptides would have higher hydrophobicity than the cyclic homochiral dipeptides. The results suggested that the separation processes of homochiral cyclic dipeptides from heterochiral cyclic dipeptides could exist in the primitive hydrosphere.

Bonner, W.A.:1991, Origins of Life **21**, 59.
Munegumi, T. and Shimoyama, A.:1996, Origins of Life **26**, 388.

P2.5

WOBBLY STEREOSPECIFICITY OF TRYPTOPHANASE IN HIGHLY CONCENTRATED SALT SOLUTION AND ITS SIGNIFICANCE FOR CHIRAL HOMOGENEITY

Akihiko Shimada
Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Chiral homogeneity has intrigued scientists since Pasteur's discovery of the optical activity of amino acids. The origin of optical activity has been discussed on the basis of abiotic chemical or physical process, but there has been still no general consensus on the question. This implies that origins of homochirality involve other factors beside the process of chemical evolution. It is enzymes that maintain present exclusive use of L-amino acids in biological world. L-dominant biological world would not have been built without the stereospecificity of enzyme, which can be related to chiral homogeneity in early metabolism. If a mechanism for chiral homogeneity had been assembled through abiotic process in primitive environment, it might have been incorporated into early polypeptides, whose descendants may also be traced to present day enzymes. High stereoselectivity in extant enzymes will reflect historical consequence of enzyme evolution. In this context, the stereospecificity of enzyme is considered to hold the key to solve the riddle of origin of homochirality. However, there is few discussion on the basis of enzymology. The reason is that the mechanism of the stereospecificity has remained unclear. Generally speaking, substrate specificity of enzyme is ample in variation from narrow to broad one. On the other hand, the stereospecificity for optical and geometrical isomers is very strict. Although enzyme active to both L- and D types of an amino acid is necessary to study a mechanism of the stereoselectivity, it is difficult to obtain such an enzyme. However, this study confirmed that tryptophanase stereospecific to L type of tryptophan became active to D-tryptophan through probably reversible steric conformational change, when TPase was exposed to highly concentrated diammoniumhydrogen phosphate solution. Such a reaction have been never reported. This reaction is practical and effective to elucidate the mechanism of the stereospecificity. We analyzed it in terms of kinetics. The advent of activity for D-tryptophan will be discussed related to chiral homogeneity.

P2.6

RIBOZYME REACTIONS AND STEREOCHEMISTRY

Ralf Stowasser and David A. Usher, Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853-1301

It has become widely accepted that ribozyme and enzyme-catalyzed displacements at phosphorus occur by an in-line mechanism, where the attacking nucleophile and the leaving group take up an in-line orientation with respect to the phosphorus (Usher, 1969). However, a few cases have been reported where the setup for displacement appears to be far from that expected for an in-line mechanism. These include a tRNA-lead complex and the hammerhead ribozyme (Pley *et al*, 1994; Scott *et al*, 1995).

It is usually assumed (Knowles, 1980) that an adjacent mechanism will result in retention of configuration at phosphorus, while an in-line mechanism will result in inversion of configuration. However, this conclusion is an over-simplification, and all that one can infer from inversion is that the leaving group and original nucleophile are in-line with the phosphorus as the trigonal bipyramid breaks down to product. In other words, adjacent *attack* can result in inversion, as long as the breakdown occurs with an in-line geometry.

We have performed high level density functional calculations that suggest that a divalent metal ion such as Mg^{2+} can stabilize an apical phosphate oxyanion, and allow adjacent attack.

Knowles, J. R. :1980, Annu. Rev. Biochem. **49**, 877.
Pley, H. W., Flaherty, K. M., and McKay, D. B. :1994, Nature **372**, 68.
Scott, W. G., Finch, J. T., and Klug, A. :1995, Cell **81**, 991.
Usher, D. A. :1969, Proc. Natl. Acad. Sci. U. S. **62**, 661.

P2.7

PARITY VIOLATING ENERGY DIFFERENCES BETWEEN ENANTIOMERS CONFIRMED BY CRYSTALLIZATION EXPERIMENTS

Andrea Szabó-Nagy and Lajos Keszthelyi
Institute of Biophysics, Biological Research Centre of the Hung. Acad. Sci. Szeged, Hungary, H-6701

The electromagnetic and weak interactions were unified by Weinberg, Salam and Glashow in the concept of the electroweak interaction. An important consequence of the theory is the existence of the neutral current that generates parity-violating interactions between electrons and electrons, and between electrons and neutrons. Experiments involving elementary particles and atoms confirmed the theory. In enantiomeric molecules, the interaction induces a small difference between the energies of the different enantiomers. The theory was extended to calculations of the parity-violating energy difference (PVED) between the L- and D-amino acids, for example. The value of PVED is $\approx 1 \times 10^{-17}$ kT (kT = 0.025 eV at room temperature), the L-amino acids having the lower energy. Despite the smallness of PVED there have been the suggestions that PVED is in fact the cause of the homochirality of biomolecules. Although the existence of a PVED between enantiomers is theoretically certain its experimental confirmation is still missing. The extremely small value of PVED probably rules out the possibility of observing it in the case of carbon-centred enantiomers. Its value, however, increases in proportion to the 6th power of the atomic number Z of the asymmetry centre. In our experiments, enantiomers with Co (Z=27) or Ir (Z=77) at the asymmetry centre: tris(1,2-ethanediamine)cobalt(III) and tris(1,2-ethanediamine) iridium(III) were used. The L and D molecules were mixed to produce racemic solutions, about half of the dissolved molecules were precipitated as polycrystalline material; aliquots were dissolved in water, their CD signals were measured with a CD spectrometer. The distributions of the CD signals for the crystalline materials (Ir and Co complexes) were fitted with Gaussians. They are shifted (in the negative direction) from the zero value and broadened relative to the distributions for the initial racemic solution and for tridistilled water. The shift for the supernatants was always positive

This study appears to have provided an experimental demonstration of the participation of the parity-violating weak interaction in addition to the electromagnetic interaction in molecules.

P2.8

FUTURE SPACE EXPERIMENT WILL CONCERN THE ORIGIN OF HOMOCHIRALITY

Wolfram H.-P. Thiemann and Uwe Meierhenrich
University of Bremen, Department of Physical Chemistry, Germany

It is common knowledge, that biological life could only arise in a homochiral environment. In this context, any questions about the origin of life are based on the models explaining the origin of homochirality.

In the 1950's the *biotic* or *selection theory* was preferred in explaining the appearance of non-racemic biomolecules in living organisms. But all experimental attempts verifying this theory failed in producing pure homochiral macromolecules in a racemic medium.

Nowadays the various models of an *abiogenic* origin of the chiral purity dating back to the prebiological chemical stage of evolution due to physical-chemical processes are more widely accepted: Here we distinguish between *random mechanisms*, which interpret the appearance of optical activity by a mere chance process, and the *determinate mechanisms*, which describe the interaction of physical driving forces with racemic substances causing the prevalence of one over enantiomer over the other. Physical driving forces on the other hand like *circularly polarized synchrotron radiation* emanated at one pole of an extremely rapidly rotating neutron star (Bonner-Rubenstein) were discussed. The effect of the universal *weak force* again resulting in a *parity violating energy difference* (PVED),is discussed in this context.

With the aim to verify these theories, ESA is planning the Cornerstone Mission ROSETTA, to visit the nucleus of the comet 46P/Wirtanen. Our laboratory participates in the development of ROSETTA'S COSAC Experiment, that is designed to identify complex organic molecules and to search for the described enantiomeric enhancement in cometary matter. One would expect far-reaching results from an identification of chiral compounds on the comet.

If the observed molecular parity violation on Earth was mainly caused by *random mechanisms* one would expect to find enantiomers of different handedness on different planetary or interstellar bodies. If homochirality was determined by *circularly polarized radiation* from a passing neutron star, all planets within a given solar system would have been affected by its same pole, and would therefore produce biomolecules of the same hand. And if biology's optical activity was determined by the universal chiral influence of the *weak force*, one would expect to find the same type of enantiomers throughout the entire universe.

P2.10

SEARCH FOR EFFECT OF WEAK-NEUTRAL-CURRENT IN THE ORIGIN OF BIOCHIRALITY

------ A NOVEL PHASE TRANSITION IN SINGLE CRYSTALS OF D- AND L-ALANINE

Wang Wenqing, Yi Fang
Department of Technical Physics, Peking University, Beijing 100871, China

Recent discoveries of excess of L amino acids in the Murchison meteorite(Engel, 1997) represent the first definite identification of exochirality and demonstrate a prebiotic chiral influence. The chiral influences that may produce biochirality can be classified into two fundamental physical fields: (1) Chiral influences (local and universal), including circularly polarized sunlight and magnetic and electric fields as well as circularly polarized radiation from neutron stars (Greenberg, 1994). (2) The universal chiral influence of the weak neutral force (mediated by the Z^0 boson) produces a very small parity-violating energy difference between enantiomers (Hegstrom, 1979). The developments in the concept of chiral interaction open new options and dynamical possibilities for the symmetry-breaking in biomolecules. However, most of them neglected the fact that such symmetry breaking can only arise when a system is far from equilibrium (Kondepudi, 1987).

In this paper we attempt to present our systematic experimental results, aiming at gaining further insight into the secondary chiral influence. Four kinds of experiments were conducted to observe the effects with the system far from equilibrium, using the condensed state of D- and L-alanine crystals. Experiments provided the following information :

(1) An obvious λ phase transition at 270±1K was shown in the specific heat measurement of alanine and valine enantiomers by differential scanning calorimetry. The biologically dominant L-enantiomer was found to have lower energy.

(2) Magnetic moment (μ) of single crystals of D- and L-alanine were measured as a function of temperature. Under a strong positive magnetic field (H) of 1 Tesla, the temperature dependant $\chi_\rho \sim T$ curves of the D- and L-alanine are different. The temperature at which the discontinuity of magnetic susceptibility occurs in D-alanine is coincident with Tc in DSC measurement. It's attributable to the variation of intramolecular geometry

P2.10 continued

of chirality density in enantiomers, which is a consequence of the differential interaction of short range WNC. The interaction energy difference between L and D, $\Delta E=2\mu H$, is on the order of 10^{-8}-10^{-10}, some 7 to 9 orders of magnitude larger than the WNC interaction energy proposed by Kondepudi et al. (1983, 1984). It can be characterized by the chiral selector factor $(\Delta E/kT)^{1/n}$, n=2,3. Under the reversed negative field of -1 Tesla, the same phenomena were observed.

(3) Laser Raman spectra of D- and L-alanine at different temperatures (100K,250K,260K,270K,280Kand 290K) showed that the second order C-H stretching frequencies at 2606cm^{-1}, 2724cm^{-1} of D-alanine vanished at 270K, but reappeared at 100K, while L-alanine has no such phenomenon. A decrease in the scattering intensity of C-H of D-alanine occurs at the λ transition point.

(4) NMR spectroscopy were recorded using Bruker DRX 300. A noticeable difference between the Carbon-13 cross polarization spectrum of D- and L-alanine is that, at 270K α carbon of D-alanine shifts to downfield, suggesting α carbon loosening its electron to undergo the observed transition; whereas in L-alanine's case, the carbonyl shifts to upfield. Possible mechanism underlying the observed phase transition is suggested.

The results are indicative of an atomic preference between left and right which arises in the period of λ phase transition in condensed state of amino acids. The observed phase transation may indicate its possible link with Salam's theory that a phase transition from a racemic mixture into an optically pure state, in which Bose-Einstein condensation probably act as an amplification mechanism, may cause the origin of biochirality (Salam, 1992). Further experiments and theoretical studies are in progress.

Engel, M.H. and Macko, S.A.: 1997, Lett. to Nature 389, 265
Greenberg, J.M.: 1994, J. Biol. Phys. 20, 61
Hegstrom, R.A.: 1979, Phys. Lett.A 71, 499
Kondepudi, D.K.:1987, Biosystems 20, 75
Kondepudi, D.K. and Nelson, G.W.:1983, Phys.Rev.Lett.50, 1023
Kondepudi, D.K. and Nelson, G.W.:1984, Phys.Lett.106A, 203
Salam, A.:1992, Phys.Lett.B 288, 153

P2.11

CONSEQUENCES AND ARTIFACTS OF AN AIR-WATER INTERFACE: TERRESTRIAL FINDINGS AND MARTIAN ANALOGUES

Louis Lerman
Nuclear Science, Lawrence Berkeley Laboratory, Berkeley, California, USA 94720 (email: Lerman@LBL.gov)

Natural consequences of the terrestrial bubble-aerosol (bubblesol) supercycle are objects with properties curiously akin to the so-named 'nanobacteria'. These properties include the basic morphology (spheres and sausages), gross chemistry (suites of organics along with metals), and size distributions (nanometers to microns).

On the contemporary Earth such objects (bubble generated aerosols and their atmospheric progeny) are the largest contributor of organic matter to the atmosphere in both particle number and total mass. This is not to say that the objects found in various martian meteorites are necessarily of such origin, but (lacking the isotopic trademarks of living systems) the superficial resemblance seems striking.

This similarity underscores the likely importance of the processes of the bubblesol cycle and their resulting mass objects with respect to the search for life and its origins within the solar system. On one hand the appearance and ubiquity of such terrestrially generated objects necessitates a deeper differentiation and categorization of life's artifacts than the coarse characteristics mentioned above.

On the other, the presence of such bubblesol objects in (or from) an extraterrestrial location suggests substantial environmental opportunities for the support of prebiotic chemical evolution. This is due to the universality of the chemical processes involved; their origin in the chemical physics of the interaction of charge-polarized organic amphiphiles with the polarity of water.

Some of the more important considerations with respect to Mars follow:

- The finding of bubblesol-generated objects (of martian origin) in martian meteorites or on Mars itself would be strong evidence for the past existence of a complex Martian hydrology cycle (one capable of gathering, concentrating, and transporting organic molecules.

P2.11 continued

- Since such a supercycle of concentration, transportation, and micro-environments may well have played a critical infrastructural role for prebiotic chemical evolution on the early Earth, indications of its existence on Mars would be most important.

- In particular, on a tectonically simpler early Mars (and one having liquid water only intermittently on its surface), such a complex hydrology cycle may well have been the most likely initiator and supporter of the rapid cycles of concentration, hydration, and dehydration most likely necessary for organic polymerization in 'bulk' quantities.

- Additionally, IF bacteria (with surface active membrane elements) have existed on Mars, and were in any way tied to a surface liquid water environment then these same bubblesol processes could have been a (and possibly the) prime mode of concentration and aerial transport. In analogy to current terrestrial processes the larger of these bubblesol-generated objects (in their hydrated state) could easily have transported such bacteria across a mostly arid planetary surface. This would have been useful for 'colonization', and could also explain the deposition and subsequent fossilization of "micro-clumps" of such bacteria in environments much removed from their origin.

Hence, any life-searching Mars missions (or interpretations of martian objects having made their way to Earth) must discriminate between the fossils of living systems of the nanobacterial scale and potential artifacts of the bubble-aerosol supercycle necessarily found on any planet having both liquid water and amphiphilic organic molecules.

The flip side to this cautionary conclusion is that the existence of such bubblesol-generated objects is highly suggestive of a complex geophysical/chemical environment capable of supporting chemical evolution. On Mars (and possibly on Earth) these processes may well have been the critical infrastructure for such chemical evolution. And even after bacterial life has been formed (wherever...) the dynamic processes of the bubblesol supercycle are likely to retain significant roles in those ecosystem processes requiring concentration and transportation at those scales driven by surface activity.

cA2.1

EVOLUTION AND SURVIVAL OF COMPLEX ORGANICS IN SPACE

B. H. Foing, P. Sonnentrucker, C. Lasseur (1)
(1) European Space Agency, ESTEC SCI-SO PB299, NL-2200 Noordwijk
P. Ehrenfreund, S. O'Tuaisrig (2), J. Cami (3), J. Krelowski (4)
(2) Leiden Observatory , (3) Amsterdam, (4) Torun Observatory

Astronomical circumstellar and interstellar observations in the UV, visible and infrared reveal the signature of complex carbonaceous material. The evolution of these complex organics from stars, interstellar medium to the early solar systems and habitable bodies, is a key to the origin of life.

We discuss the evidence for complex organics in the interstellar medium from their spectral signature such as UV extinction, Diffuse Interstellar Bands and Aromatic Infrared Emission Bands. We review new information on the carriers of the visible Diffuse Interstellar Bands. Evidence for large molecules with 30-70 C atoms was shown for several DIBs (Ehrenfreund & Foing 1996). A statistical study indicates that carriers of DIBs are distinct molecules, though some show a family behaviour (Cami et al. 1997). A new survey of diffuse interstellar bands reports a population of DIBs molecules enhanced in specific lines of sight (O' Tuaisrig et al. 1999). Correlations with simple molecular species such as CH or CH^+ (Krelowski et al. 1999) or atomic species (Sonnentrucker et al. 1999) indicate where DIB carriers reside in interstellar clouds. New information was derived on ionisation (Sonnentrucker et al. 1997) and double-ionisation properties which determine the physical state and survival of these species.

We discuss relevant laboratory and theoretical studies stimulated by related astronomical observations. Recent results are described on the search for specific Polycyclic Aromatic Hydrocarbons, long carbon chains, fullerenes (Foing & Ehrenfreund 1994, 1997), fulleranes and derived compounds. We discuss how the abundances and signatures of these molecules vary in different interstellar and circumstellar environments.

We describe constraints on the formation, evolution, ionisation, destruction, survival of these complex organics in the interstellar medium. The distribution of PAHs and fullerenes measured in meteorites was compared to that derived for interstellar environments (Foing et al. 1999). Our "Organic Matter" experiment selected to fly on the International Space Station Exposure Facility to UV, cosmic ray, and vacuum conditions will further investigate the evolution of complex organics in space. Finally, we discuss some open questions on the transport and delivery of these complex organics into the solar system and onto Earth.

Cami, J., Sonnentrucker, P., Ehrenfreund, P., Foing, B.H.: 1997, A&A **326**, 822.
Ehrenfreund, P. and Foing, B.H. : 1996, A&A **307**, L25.
Foing, B.H. and Ehrenfreund, P. : 1994, Nature **369**, 296
Foing, B.H. and Ehrenfreund, P.: 1997, A&A **317**, L59
Foing, B.H. et al. : 1999, in preparation.
Krelowski, J. Ehrenfreund, P.., Foing, B.H. et al. : 1999, A&A in press
Sonnentrucker, P., Cami, J., Ehrenfreund, P., Foing, B.H.: 1997, A&A **327**, 1215.
Sonnentrucker, P., Foing, B., Breitfellner, M., Ehrenfreund, P.:1999, A&A in press

cA2.2

EXPERIMENTAL SIMULATION OF THE PHOTODEGRADATION OF LARGE ORGANIC MOLECULES IN COMETARY ENVIRONMENT

H. Cottin, M.C. Gazeau, J.F. Doussin, F. Raulin
LISA, U.M.R. 7583 CNRS, Université Paris XII, 94000 Créteil, France

We present a current experimental program concerning the study of the photochemical evolution of the organic matter ejected from the cometary nucleus surface : S.E.M.A.Ph.Or.E. Cométaire (a french acronym : Simulation Experimentale et Modelisation Appliquées aux PHénomènes ORganiques dans les Environnements Cométaires). The aim of the work is to understand better, using laboratory simulations, the mechanisms which are involved during the degradation of the high molecular weight organics in cometary ices and dust when they are submitted to the warming up and to the bombardment of photons in the surrounding area of the sun. This experimental study will establish correlations between the nucleus and the coma's molecular composition. Furthermore, experimental data will provide useful information to bring to a close the question of the origin of the extended sources such as H_2CO and CN.

Polyoxymethylene (POM), suspected to be present in the cometary nucleus, is often mentioned as a possible parent molecule for the formaldehyde's extended source, but the key physico-chemical data are missing to conclude. To test this hypothesis, irradiation of POM has been performed at 147 nm (Xenon Lamp) and 122 nm (Hydrogen Lamp) in order to obtain the quantum yield of production, from the Polyoxymethylene, of the photodegradation products.

Results on the photodegradation of Hexamethyltetramine (HMT), which could be a parent molecule of the CN radical, will also be presented.

cA2.4

THE COSMOGEOCHEMISTRY OF AMINO ACID SYNTHESIS FROM HYDROGEN CYANIDE

K. L. F. Brinton[1], G. D. McDonald[1], J. P. Dworkin[2], M. Levy[3], S. L. Miller[4] and J. L. Bada[5]

[1]Jet Propulsion Laboratory, MS 183-301, Pasadena, CA 91109, [2]NASA Ames Research Center, MS 245-6, Moffett Field, CA 94035, [3]Department of Molecular Biology, The University of Texas at Austin, Austin, TX 78712, [4]Department of Chemistry and [5]The Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093

The synthesis of amino acids from hydrogen cyanide (HCN) may provide important insights into the prebiotic chemistry of comets, the primitive Earth and the oceans on Jupiter's moon Europa. A series of hydrogen cyanide (HCN) polymerization reactions were carried out at various temperatures, pH values and HCN concentrations. The reaction solutions were hydrolyzed in 6 N HCl and analyzed for amino acids using high performance liquid chromatography with fluorescence detection. Glycine was found to be the main amino acid product and accounted for 75-98% of the total amino acids produced. Other amino acids detected were alanine, aspartic acid, and possibly diaminosuccinic acid. The synthesis of glycine as a function of time and the effects of the various reaction conditions on the rate and maximum yield of glycine production will be presented.

Two frozen cyanide solutions, which had reacted over a period of 25 years, were also analyzed and their glycine production compared with reactions taking place at higher temperatures in liquid water. The glycine yields of the frozen solutions were similar to those in higher temperature liquid reactions, suggesting that even in cold environments, the acid-labile precursors of glycine could be readily produced from HCN, possibly as a result of eutectic concentration of aqueous HCN.

The distinctive distribution of amino acids produced directly from HCN products will be discussed as a possible means of identifying the origin of amino acids in cosmogeochemical samples.

cA2.5

SIMULATIONS OF COMETARY ICE: LARGE MOLECULE SYNTHESIS AND SELF-ASSEMBLY PROPERTIES

Jason P. Dworkin[*†], Scott A. Sandford[*], Louis J. Allamandola[*], David W. Deamer[‡], J. Seb Gilette[¶], and Richard N. Zare[¶]

[*]Astrochemistry Laboratory, NASA Ames Research Center 245-6, Moffett Field CA, USA 94035-1000
[†]SETI Institute, 2035 Landings Dr., Mountain View CA, USA 94043
[‡]Department of Chemistry and Biochemistry, UCSC, Santa Cruz CA, USA 95064
[¶]Department of Chemistry, Stanford University, Stanford CA, USA 94305-5080

The combination of realistic laboratory simulations and infrared observations have revolutionized our understanding of interstellar dust and ice–the main building blocks of comets. Since comets and carbonaceous micrometeorites may have been important sources of volatiles and carbon compounds on the early Earth, their organic composition may be related to the origin of life (Thomas et al., 1997). Ices on grains in molecular clouds contain a variety of simple molecules (Allamandola et al., 1997). Within the cloud and especially in the presolar nebula, these icy grains would have been photoprocessed by the ultraviolet producing more complex species such as hexamethylenetetramine, polyoxymethylenes, and simple keones (Bernstein et al., 1995).

Here we report laboratory simulations studied to identify the types of molecules which could have been generated in pre-cometary ices. Experiments were conducted by forming a realistic interstellar mixed-molecular ice (H_2O, CH_3OH, NH_3, and CO) at ~10 K under high vacuum irradiated with UV from a hydrogen plasma lamp.

The residue that remained after warming to room temperature was analyzed by HPLC, and by several mass spectrometric methods. This material contains a rich mixture of complex compounds with mass spectral profiles resembling those found in IDPs and meteorites. Surface tension measurements show that an amphiphilic component is also present. These species do not appear in various controls or in unphotolyzed samples.

Residues from the simulations were also dispersed in aqueous media for microscopy. The organic material forms 10-40 μm diameter droplets that fluoresce at 300-450 nm under UV excitation. These droplets appear strikingly similar to those produced by extracts of the Murchison meteorite (Deamer, 1985).

Together, these results suggest a link between organic material photochemically synthesized on the cold grains in dense, interstellar molecular clouds and compounds that may have contributed to the organic

inventory of the primitive Earth. For example, the amphiphilic properties of such compounds permit self-assembly into the membranous boundary structures that required for the first forms of cellular life.

Bernstein, M.P., Sandford, S.A., Allamandola, L.J., Chang, S., and Scharberg, M.A.:1995, Ap. J. **454**, 327.
Deamer, D.W.:1985, Nature **317**, 792.
Allamandola, L.J., Bernstein, M.P., Sandford, S.A.: 1997, Proc. 5th Int. Conf. on Bioastronomy, IAU Coll. #161, Capri, 1-5 July 1996, (Editrice Compositori: Bologna), pp. 23-47.
Thomas, P.J., Chyba, C.F., and McKay C.P.: 1997, *Comets and the Origin and Evolution of Life*, Springer, New York.

cA2.6

ABIOGENIC SYNTHESIS OF GUANINE NUCLEOTIDES UNDER THE ACTION OF VACUUM ULTRAVIOLET

E.A.Kuzicheva, M.B.Simakov, N.B.Gontareva and P.V. Semenchenko
Institute of Cytology, Russian Academy of Sciences, Tikhoretsky pr., 4, 194064 StPetersburg, Russia

The abiogenic synthesis of guanine nucleotides under the action of vacuum ultraviolet radiation (VUV) as one of the most efficient open space source of energy has been studied. Formation of mononucleotides from certain nucleosides after the exposure of guanosine, deoxyguanosine, phosphate and NaH_2PO_4 dry films has been analyzed. 5'-guanosinemonophosphate and 5'-deoxyguanosinemonophosphate were the main products obtained. Maximum VUV induced yield of nucleotides in relation to irradiation dose (λ=145 nm) accounts for 0.30% (5'GMP); 0.27% (2',3' cGMP); 0.22% (3'GMP); 0.21% (2'GMP); 0.05% (3',5'cGMP); 0.23% (5'dGMP); 0.13% (3'dGMP); 3',5' cdGMP exists in small proportion. Identification and quantitative evaluation of synthesized and degradated products were carried out by means of HPLC method.

The results obtained will be discussed in accordance with their significance in terms of further Earth orbit exobiological investigations. At present time it is generally recognized that such an investigations could lead to a better understanding of the processes of life origin and evolution on Earth or elsewhere in the Universe.

cA2.7

AN EXPERIMENTAL STUDY OF THE SHOCK REACTIVITY AND STABILITY OF COMETARY ORGANIC MATTER

J. G. Blank, University of California, Berkeley CA 94720
G. H. Miller, University of Chicago, Chicago IL 60637
R. E. Winans, Argonne National Laboratory, Argonne IL 60439

We have developed an experimental method to test the viability of extraterrestrial delivery of organic compounds via cometary impacts. Our goal is to determine decomposition kinetics scalable to large natural impacts.

Using an 80 mm cannon, a stainless steel flyer plate is propelled down a 12 m barrel at velocities of 0.5-2.5 km/s by burning gunpowder. The plate impacts a stainless steel container, which contains ≈40 μl of organic solution. This target is thrown backward into a deceleration medium and recovered intact. We then drill into the container, extract the recovered solution, and analyze the run products using liquid and gas chromatography mass spectrometry.

Our initial experiments have used lysine, a common terrestrial amino acid, and norvaline, an amino acid present in Murchison meteorite. These two compounds are readily soluble in water and have different thermal stabilities. They are chemically similar, although lysine has additional functionality due to its extra amino group. Small amounts of both amino acids survive the shock heating, and lysine appears to be more resilient to breakdown. Small amounts of decarboxylation products were also recovered from the shocked samples.

We plan to vary the maximum pressure the sample experiences by varying the impact velocity, and vary the duration of the peak pressure by changing the thickness of the sample container. These are the first experiments of this kind to determine the stability of aqueous solutions of amino acids subjected to such extreme conditions. With the combined well-characterized, pressure-temperature-time trajectories of our experiments, we will determine kinetic rate constants and activation volumes of our selected compounds. Our results will be used in a quantitative estimate of the organic carbon flux to the Earth by comets based on laboratory measurements.

cA2.8

FULLERENES AND THE FLUX OF EXTRATERRESTRIAL HELIUM ($HE@C_{60}$) TO THE EARTH DURING GIANT IMPACT EVENTS.

Luann Becker[1], Robert Poreda[2] and Ted Bunch[3], [1]University of Hawaii, Honolulu, HI 96822, tel. 808-956-3188; [2]Rochester University, Rochester, NY 14627, [3]NASA Ames Research Center, Moffet Field, CA 94035-1000

The discovery of fullerenes in deposits associated with two separate impact events involving a large bolide with the Earth raises the possibility that these carbon (C) molecules may also be an indicator of ET events over geologic time (Becker et al., 1998). Fullerenes were detected in carbon-rich breccias (Onaping Formation) associated with the 1.85 billion-year-old Sudbury Crater (Becker et al., 1994) and in clay sediments within the 65 million-year-old Cretaceous/Tertiary (K/T) boundary (Heymann et al., 1994). In an effort to determine the origin of the Sudbury fullerenes, we searched for noble gases trapped inside the fullerene molecules (Saunders et al., 1993). The result of this study revealed that the Sudbury fullerenes contain trapped $^3He/^4He$ ratios (~5.5 X 10^{-4}) that are similar to those found in meteorites and some interplanetary dust particles (Becker et al., 1996). Based on these results it was suggested that fullerenes may be exogenously delivered to the Earth in some meteorites or comets and that fullerenes may also be a unique carrier of noble gases in certain astrophysical environments.

Recently, we have begun investigating the possibility of ET helium in fullerenes isolated from sediments in the 65 myr old K/T boundary. Preliminary measurements of helium in a continental K/T boundary fullerene residue from the Raton basin (Madrid site, Colorado) revealed $^3He/^4He$ ratios some 100 times above air. The concentration of helium (per μg of fullerene) in K/T fullerenes (5 μg sample) is within a factor of two of the helium concentration in the Sudbury fullerenes (~100 μg) with a similar temperature release pattern. A marine K/T boundary residue from Stevns Klint, Denmark, analyzed for helium, revealed $^3He/^4He$ ratios several thousand times above air in the high temperature fraction! The concentration of helium (per μg of fullerene) in Stevns Klint (~8 μg sample) indicates that the ^3He concentration is some 4 times higher than that found in the Sudbury fullerenes. We attribute the anomalously high $^3He/^4He$ ratios and high ^3He concentration for the Stevns Klint K/T sample to the abundance of higher fullerenes extracted from the residue. The high $^3He/^4He$ ratio in the K/T fullerenes suggests that they were present in the bolide and somehow survived the impact event. Confirmation of these results could have broad implications concerning the importance of large impacting bolides in providing ET helium and perhaps other volatiles and organics to the Earth's crustal reservoir.

Becker et al., 1994, *Science* **265,** p. 642, Heymann et al., 1994, *Science* **265,** p. 645. Saunders et al., 1993, *Science* **259,** 1428. Becker et al., 1996, *Science* **272,** p. 249.

cA2.9

ORGANIC COMPOUNDS IN THE K/T BOUNDARY SEDIMENTS AT KAWARUPPU, JAPAN AND THEIR COMPARISON WITH THOSE IN THE CARBONACEOUS CHONDRITES.

Hajime Mita, Hikaru Yabuta and Akira Shimoyama
Department of Chemistry, University of Tsukuba, Tsukuba 305-8571, JAPAN

Finding of α-aminoisobutyric acid and isovaline in the K/T boundary sediments at Stevns Klint is related to bolide impact at the end of Cretaceous (Zhao and Bada, 1989). We failed to detect the two amino acids in the K/T boundary sediments at Kawaruppu, Japan (Mita, *et al.*, 1996a). Therefore, we analyzed dicarboxylic acids and hydrocarbons in the K/T boundary sediments and compared them with those in the carbonaceous chondrites.

Twenty-seven dicarboxylic acids, 60 aliphatic hydrocarbons, and 26 aromatic hydrocarbons were detected in the K/T boundary sediments at Kawaruppu. Dicarboxylic acids included normal (C_2 to C_8), branched (C_4 to C_7) and unsaturated (C_4 and C_5) forms. Aliphatic hydrocarbons included n-alkanes (C_{12} to C_{36}), pristane, phytane, alkylcyclopentanes (C_{12} to C_{28}), alkylcyclohexanes (C_{11} to C_{26}), decahydronaphthalene, and adamantane. Aromatic hydrocarbons were from naphthalene to coronene and their alkyl derivatives.

We did not find any indicator compound(s) of extraterrestrial origin among those dicarboxylic acids and hydrocarbons in the K/T sediments at Kawaruppu. In addition, we failed to find a good correlation between the sediments and the meteorites. The molar ratios of normal to normal plus branched dicarboxylic acids in all the samples are clearly higher than those in the Murchison and Yamato-791198 meteorites (Shimoyama and Shigematsu, 1994). The ratios are rather similar to those in the Neogene Shinjo sediments (Mita, *et al.*, 1996b), although it is possible that an extraterrestrial contribution is hidden by an overlay of terrestrial organic compounds in the sediments.

Mita, H., Shimoyama, A. and Kajiwara, Y.:1996a, *Geochem. J.* **30**, 89.
Mita, H., Shigematsu,R. and Shimoyama, A.:1996b,*Geochem.J.***30**,251.
Shimoyama A. and Shigematsu, R.:1994, *Chem. Lett.* **1994**, 523.
Zhao, M. and Bada, J. L.:1989, *Nature* **339**, 463.

cB2.1

A PHOTOCHEMICAL FLOW REACTOR FOR THE LABORATORY STUDY OF ATMOSPHERIC CHEMISTRY: APPLICATION TO TITAN

Jeffrey C. Joseph, David W. Clarke and James P. Ferris
Dept. Of Chemistry, Rensselaer Polytechnic Inst., Troy, NY, USA 12180

TITAN

Titan, the largest moon of Saturn, has a 1.5 bar atmosphere consisting of about 98% N_2 and 2% CH_4. Smaller amounts of higher hydrocarbons and nitrogen-containing organics are also present. Haze layers in the atmosphere make it impossible to see the surface of this moon. It is likely that the haze is formed by the photochemically-initiated polymerization of the unsaturated compounds present in the atmosphere of Titan.

We have undertaken a series of laboratory studies of the photochemistry of cyanoacetylene (HC_3N) - acetylene (C_2H_2) mixtures admixed with other compounds present in Titan's atmosphere to probe the formation of the haze and other atmospheric constituents. This study will contribute to the understanding of the data obtained from the Huygens probe of the Cassini orbiter due to reach Titan in 2004. Since it is likely that photochemical transformations were important in the chemical evolution of organics in the atmosphere of the primitive Earth, these studies may provide insights into the atmospheric processes there. (Clarke and Ferris 1977a).

PHOTOCHEMICAL FLOW REACTOR

Previous studies of HC_3N photochemistry in our laboratory have examined the primary as well as tsecondary photochemical processes. (Clarke and Ferris, 1995, 1996, 1997b) The studies were performed using pressures of HC_3N >1 torr. While this research provided insight into the photochemical processes on Titan, it was never certain that the results could be extrapolated to Titan where the mixing ratio of most constituents is 10^{-5}-10^{-7}. The use of a lower pressure of HC_3N in a static system is not practical as neither the small amount of reactant consumed nor product formed can be determined accurately.

A flow system was designed to overcome the limitations of static systems. The flow reactor has four important advantages over static systems in the laboratory investigations of photochemical processes of trace components in a gas mixture. First, measurable amounts of minor constituents can be diluted with a major gas to achieve the mixing ratios present in the atmosphere of the planet or moon under investigation. A second advantage is that the time of photolysis and the flow rate can be varied to obtain enough reaction products for analysis. The third advantage

cB2.1 continued

(Photochemical flow reactor, continued)

is that the composition of the gas mixture being photolyzed does not vary with time because fresh reactant gas is always flowing into the irradiation zone of the cell. This is a much better model of a satellite's or planet's photochemical reactions than a static reactor since only a small percent of the atmosphere is being photolyzed at one time. The flow system also removes the photoproducts from the irradiation zone, a process which minimizes the formation of secondary photoproducts. Fourth, the very low partial pressures of the reactants results in their absorption of less than 1% of the incident light. This results in photochemistry throughout the volume of the irradiation chamber and not just near the walls. Along with the high pressure of the inert gas, this greatly decreases the possibility that reactions on the walls would give products that would not form in the atmosphere.

The flow system was used to further probe the atmospheric chemistry of Titan. In this research mixtures of N_2, CH_4, H_2, C_2H_2,, C_2H_4 and HC_3N, in which the mixing ratios were comparable to those on Titan (0.98, 0.018, 2 x 10^{-3}, 3.5 x 10^{-6} 3.0 x 10^{-6} and 10^{-7}, respectively) were irradiated and the volatile and solid products were collected and analyzed. The volatile products were analyzed qualitatively and quantitatively by infrared and nuclear magnetic resonance spectroscopy. The rates of the loss of reactants and the formation of products (quantum yields) provide quantitative data on the photochemical efficiencies of these processes in Titan's atmosphere. The solid products formed (haze particles) were collected and the structural units present were determined by infrared spectroscopy. The light absorption properties of the solids were analyzed by UV-visible spectroscopy and the imaginary index of refraction was determined from these measurements. The size distribution and morphology of the particles formed were determined by scanning electron microscopy. This research provides direct information as to the source of some of the volatiles in the atmosphere of Titan as well as the structure of the Titan haze.

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Clarke, D. W. and Ferris, J. P., Icarus, 1995, **115**, 119-125
Clarke, D. W. and Ferris, J. P.:1996, J. Geophys. Res., E3, **101**, 7575-7584.
Clarke, D. W. and Ferris, J. P.: 1997a, Origins Life Evol. Biosphere **27**, 225-248.
Clarke, D. W. and Ferris, J. P.: 1997b, Icarus, **127**, 158-172

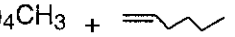
cB2.2

ORGANIC PHOTOHEMISTRY IN THE ATMOSPHERE OF JUPITER AND SATURN. THE ROLE PLAYED BY H_2S, PH_3 AND NH_3.

Jean-Claude Guillemin
Laboratoire de Synthèse et Activation de Biomolécules, ENSCR, 35700 Rennes, France.

Several hydrocarbons and heterocompounds have been detected in the atmospheres of Jupiter and Saturn. Billion years of Solar illumination have probably produced numerous minor components. We present here an experimental work devoted to the photolysis of NH_3, PH_3 or H_2S in the presence of an alkene (ethene) or an alkane (methane, ethane).
The major products formed in the photolysis of one of these heterocompounds and ethene are the corresponding heteroalkanes. However, the presence of minor products clearly shows that the mechanism of each reaction is different.

$$PH_3 + C_2H_4 \xrightarrow[185\ nm]{h\nu} H_2PCH_2CH_3 + H_2C{=}CHPH_2 + \ldots$$

$$NH_3 + C_2H_4 \xrightarrow[185\ nm]{h\nu} H_2NCH_2CH_3 + H_2N(CH_2)_3CH_3 + H_2N(CH_2)_4CH_3 + \text{  } + \ldots$$

$$H_2S + C_2H_4 \xrightarrow[254\ nm]{h\nu} HSCH_2CH_3 + EtSSEt + \ldots$$

The photolysis of NH_3, PH_3 or H_2S in the presence of an alkane led to the corresponding amine, phosphine or thiol when an appropriate ratio of the two gases was used. Reaction pathways for such reactions are proposed.

$$PH_3 + C_2H_6 \xrightarrow[185\ nm]{h\nu} C_2H_5PH_2 + P_2H_4 + \ldots$$

$$H_2S + C_2H_6 \xrightarrow[254\ nm]{h\nu} C_2H_5SH + \ldots$$

The relevance to the photolysis of the atmosphere of Jupiter and Saturn is discussed.

Guillemin, J.-C.; Breneman, C. M.; Josepha, J. C.; Ferris, J. P.; *Chemistry, Eur. J.*; 1998, *5*, 1074-1082.
Mó, O.; Yáñez, M.; Decouzon, M.; Gal, J.-F.; Maria, P.-C.; Guillemin, J.-C.; *J. Am. Chem. Soc.*, 1999, *in press.*

cB2.3

IN SITU ANALYTICAL TECHNOLOGY FOR EXOPALEONTOLOGY

Thomas J. Wdowiak and David G. Agresti
Astro & Solar System Physics Program
Department of Physics
University of Alabama at Birmingham
Birmingham, AL 35294-1170 USA

The advent of instrument suite capable rovers having also the capability for acquiring fresh surfaces of rocks during the sampling process, offers opportunity for exopaleontological investigations as a component of Mars exploration. The perceived role for these instruments is in prospecting for mineralogies indicative of potential ancient habitats, and specifically for selection of samples capable of preserving relict evidence including fossils. Importantly this is a role that is in addition to that focused on planetary science goals, eliminating issues that arose for the Viking missions.

Studies in our laboratory reveal direct application for Mossbauer and Raman spectrometers (to be deployed during the Athena Mars missions) in identifying minerals indicative of aqueous activity including hydrothermal systems, and harboring carbonaceous residues of ancient (terrestrial Archean and Proterozoic) bacteria. This includes definitive spectral signatures of sedimentary minerals such as carbonates, sulphates, hydrothermal silica, hydrothermal nanophase iron oxyhydroxides, and carbon species derived from biology and encapsulated in cherts (i.e. Fig Tree, Gun Flint) and impact breccia (Sudbury). Spectral signatures will be presented and issues surrounding the acquisition process, including constraints for sample interrogation will be discussed.

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cB2.4

CARBON ISOTOPES AS A BIOGEOCHEMICAL TOOL : EVOLUTION OF A CONCEPT

Manfred Schidlowski
Max-Planck-Institut für Chemie, Postf. 3060, D-55020 Mainz, Germany

Organisms synthesize biological substances with (1) a high degree of structural specificity, and (2) a limited distribution of structural types. This consequently results in a high degree of order in biogenic matter that is manifest on both the intra- and intermolecular levels. Since the pioneering work by Nier and Gulbransen (1939) it is firmly established that preferential isotopic arrangements are also part of this ordered state. In fact, the marked bias for ^{12}C in organic materials that primarily derives from an enzymatic (kinetic) isotopic effect imposed on the first C-fixing carboxylation reaction of the assimilatory pathway has turned out to be one of the most durable and sturdy relics of the ordered state of the primary biological substances that may be preserved in sedimentary organics over billions of years.

Since the mid-70's, an impressive data base has been assembled, documenting that biologically mediated carbon isotope fractionations have ostensibly persisted over 3.8 Ga of recorded Earth history. Specifically between 3.5 Ga ago and the present, the mainstream of the $\delta^{13}C$ age curve of sedimentary organic carbon ("kerogen") can be best interpreted as the geochemical manifestation of the isotope-discriminating properties of the key enzyme of the Calvin cycle (ribulose-1,5-bisphosphate carboxylase) that channels most of the carbon transfer from the nonliving to the living world. A residual problem of the carbon isotope record is the apparent resetting of the $\delta^{13}C_{org}$ age function in the Earth's oldest sediments from Isua, West Greenland, which had been subjected to amphibolite-grade metamorphism.

Ever since the assemblage of the first comprehensive sets of Isua data (Schidlowski et al., 1979, 1983; Hayes et al., 1983), a whole critical industry had grown up around the subject which, however, notoriously ignored the fact that chemical reactions in metamorphic systems are not governed by chaos, but by well-constrained physicochemical equilibria. It is firmly established today that $^{13}C/^{12}C$ exchange can occur in kerogenous and graphitic rock constituents during both amphibolite and granulite facies metamorphism if there is a second carbon partner around (in the form of

cB2.4 continued

either fluids or carbonate). Sometimes complete isotopic reequilibration may be achieved, but often the exchange is only partial due to sluggish kinetics in the reduced carbon constituents. In any case, thermodynamic equilibria predict that $^{13}C/^{12}C$ ratios in kerogen and graphite increase during this process. Hence, high-T exchange equilibria are always bound to push $\delta^{13}C$ in sedimentary organics towards more positive values (and never in negative direction). Thus, the lowermost values encountered in metamorphosed organics are always the least exchanged and most pristine (Valley, 1986). Since the lowermost $\delta^{13}C$ values of reduced (graphitic) carbon encountered in the early Isua surveys fell into the range -22 to -28‰ [PDP], this constituted straightforward evidence that carbon constituents with the isotopic composition of biogenic matter had been indeed present in the pre-metamorphic Isua rocks. It was, therefore, by no means surprising that the results of recent isotope work based on highly sophisticated techniques of instrumental microanalysis (Mojzsis et al., 1996) has prompted similar conclusions.

Hayes, J.M., Kaplan, I.R., Wedeking, K.W.: 1983, in J.W. Schopf (Ed.), *Earth's Earliest Biosphere: Its Origin and Evolution* (Princeton U.P.), 93-134.
Mojzsis, S.J., Arrhenius, G., McKeegan et al.: 1996, Nature **384**, 55-59.
Nier, A.O., Gulbransen, E.A.: 1939, J. Am.Chem. Soc. **61**, 697-698.
Schidlowski, M., Appel, P.W.U. et al.: 1979, Geochim. Cosmochim. Acta **43**, 189-199.
Schidlowski, M., Hayes, J.M., Kaplan, I.R.: 1983, in J.W. Schopf (Ed.), *Earth's Earliest Biosphere: Its Origin and Evolution* (Princeton U.P.), 149-186.
Valley, J.W.: 1986, in Valley et al. (Eds.), *Stable Isotopes in High-T Geological Processes* (MSA Rev. Miner. **16**), Min. Soc. Am., Washington D.C., 445-489.

cB2.5

AN EARLY ARCHEAN, ORGANIC CARBON-RICH MICROBIALITE (3.3-3.4 GA) FROM THE BARBERTON GREENSTONE BELT, SOUTH AFRICA

Frances Westall[1], Maarten J. de Wit[2], Maud M. Walsh[3], Robert L. Folk[4], Chafetz, H.[5], and Everett K. Gibson, Jr.[1]

[1] SN2-NASA-Johnson Space Center, Houston TX 77058, USA, frances.westall1@jsc.nasa.gov; [2] Dept. Earth Sciences, Univ. Cape Town, Rondebosch, 7700 Cape, South Africa; [3] Institute for Environmental Studies, Lousiana State Univ., Baton Rouge, LA 70803, USA; [4] Dept. Geological Sciences, Univ. Texas at Austin, Austin, TX 78712, USA; [5] Dept. Geosciences, Univ. Houston, TX 77204-5503, USA.

The Buckridge Chert horizon (3.440 -3.445 Ga) occurs at the top of the Hooggenoeg Formation (Onverwacht Group of the Swaziland Supergroup) and yields small but macroscopically identifiable microbialites containing graphitised organic carbon. These microbial buildups- complement previous observations of microfossils and microbialites from the other chert horizons within the Onverwacht Group (Walsh, 1992; Westall et al., in press). They demonstrate the diversity of microbial structures and microorganisms already present in the Early Archean.

Chert horizons in the Onverwacht Group are interbedded with volcanics and volcaniclastics. The silicified deposits of the Buckridge Chert horizon were deposited in a stable tectonic regime (Worrell, 1985; Walsh, 1992). Field relationships suggest that the horizon formed in shallow waters. The presence of delicate, "cauliflower"-shaped microbial buildups, draped by graded layers of ash, indicates formation in a quiet hydrodynamic regime. The ash coatings and apparently syngenetic silicification testify to ongoing volcanic and hydrothermal activity as the microbialites developed.

The microbial structures are complex, exhibiting a variety of sizes and shapes. The largest are centimeter-sized, gravity-defying, growths having either a hummocky or "cauliflower" shape. They also form sub-horizontal, undulating layers 100s of microns in thickness. The mini-

stromatolites are outlined by dark layers, intercalated with lighter, relatively clean horizons of chert, producing fine, black and white-alternating laminae on a scale of 100s of microns. The dark layers consist of fine, filigree-structured filaments of dark, carbonaceous matter. Beneath the "humps" of the ministromatolites are clouds of dark, carbonaceous matter. This carbonaceous matter represents the graphitised remains of the organisms that constructed the microbialites. They are not, however, microfossils. [The organic carbon from the decomposed organisms has been remobilised and converted to graphite by lower greenschist metamorphism.] Stable carbon isotope measurements will provide more information about the nature of the carbonaceous matter.

The similarity in structure and texture of this Early Archean sample to both modern and ancient stromatolitic travertines is striking (Chafetz and Folk, 1984). The gravity-defying growths and orientations of the shrubs and filaments may indicate a microbial phototactic strategy as in modern travertines (Chafetz and Folk, 1984).

Chafetz, H.S. and Folk, R.L.: 1984, J. Sediment. Petrol., **54**, 289.
Walsh, M.M.: 1992, Precambrian Res. **54**, 271.
Westall, F., de Wit, M., Dann, J., van der Gaast, S., de Ronde, C.E.J and Gerneke, D.:in press, Precambrian Res.
Worrell, G.F.:1985, *Sedimentology and mineralogy of silicified evaporites in the basal Kromberg Formation, South Africa*, MA thesis, Louisiana State Univ.

cB2.6

MICROBIAL GROWTH RATES IN PERMAFROST DOWN TO -20°C.

E. I. Friedmann,[1] E. M. Rivkina, [1,2] C. P. McKay,[3] D. A. Gilichinsky[2]

[1]Florida State Univ., Tallahassee, FL 32306-1100, USA, FAX: 1-850-644-9829, e-mail: friedm@bio.fsu.edu; [2]Inst. of Basic Biol. Problems, Russian Acad. Sci., Puschino, Moscow Region 142292, RUSSIA, FAX: 7-096-779-0532, e-mail: gilichin@issp.sherpukov.su; [3]NASA-Ames Res. Ctr., Mail Stop 245-3, Moffett Field, CA 93035, USA, FAX: 1-415-604-6776, e-mail: cmckay@mail.arc.nasa.gov.

Siberian permafrost harbors large numbers of viable microorganisms for up to 3 million years and Antarctic permafrost probably for much longer. Metabolic activity was measured in a natural population of bacteria in Siberian permafrost (permanently frozen soil) between +5 and -20°C, on the basis of incorporation of ^{14}C-labeled acetate into lipids. Incorporation followed the usual sigmoidal growth pattern. At all temperatures, log phase was followed, within 200-350 days, by a stationary phase, monitored until the 550th day of activity. Doubling times ranged from 1 day (+5°C) to 20 days (-10°C) to ca. 160 days (-20°C). The curves reached stationary phase at different levels, depending on incubation temperature. There is evidence that the stationary phase, generally considered to be reached when the availability of nutrients becomes limiting, is brought on here by the formation of diffusion barriers in the thin layers of unfrozen water known to be present in permafrost soils, the thickness of which depends on temperature.

These results suggest that Martian permafrost, at a depth where temperatures reach at least -20°C, is a potential habitat for microorganisms, which may be able to survive there for millions of years.

cB2.8

THE HOMOPOLYMER PROBLEM IN THE ORIGIN OF LIFE

Robert Shapiro, Department of Chemistry, New York University, New York, N.Y. 10003, USA. E-mail: rs2@is2.nyu.edu

A number of theories hold that life began with the spontaneous formation of a polymeric organic replicator within an unorganized chemical mixture. The theories differ on the identity of the replicator, but they specify that it has been constructed from a mixture of subunits of a particular chemical class; the assembled polymer contains a uniform backbone which carries attached, variable, information-bearing subunits. Among the candidates proposed for the first replicator have been RNA, DNA, proteins, and peptide nucleic acid (PNA) (Miller, 1997); many others have been suggested (Joyce, *et al.*, 1987; Schwartz, 1997).

Little attention has been paid to one difficulty in obtaining such a polymer, however. Prebiotic chemical mixtures are likely to have contained a host of chemical substances, many of which would compete as the next to be inserted in any undirected polymerization process. As an example, we will consider the likelihood of getting a polypeptide made entirely of L-α-aminoacids through a polymerization involving the components of the Murchison meteorite (Cronin, *et al.*, 1988). The D-enantiomers would compete for incorporation, as would numerous β-aminoacids and hydroxyacids. Monofunctional carboyxlic acids (much more abundant than amino acids in Murchison) would tend to terminate any chain on one end, and aliphatic amines (as prevalent as amino acids) would do so on the other. Trifunctional substances such as aspartic acid would introduce branches. Similar, or even more profound, difficulties arise in the spontaneous assembly of the other organic replicators proposed for origin of life. Problems of this type have been avoided in many prebiotic simulations by the exclusion of competing substances from the polymerization mixture. On the early Earth, the production of an information-bearing homopolymer by chance within a complex mixture cannot be excluded, but if such an event was required to start life, then the origin would have been an extremely improbable accident.

Some possibilities which avoid this difficulty deserve further attention. A mineral may have served either as the first replicator or as a highly selective polymerase. Another alternative is that life began as a metabolic network of reactions involving monomers, and that a replicator came later.

cB2.8 continued

THE HOMOPOLYMER PROBLEM IN THE ORIGIN OF LIFE

Cronin, J.S., Pizzarello, S. and Cruikshank, D.P.:, 1988, in *Meteorites and the Early Solar System,* Kerridge, J.F. and Matthews, M.S., eds., Univ. of Arizona Press, Tucson, 819-857.
Joyce, G.F., Schwartz, A.W., Miller, S.L. and Orgel, L.E.: 1987, Proc. Natl. Acad. Sci. USA **84**, 4398.
Miller, S.L.: 1997, Nature Structural Biol. **4**, 167.
Schwartz, A.W.: 1997, J. theor. Biol. **187**, 523.

i3.1

SPREAD AND MORE: SELECTION OR COEXISTENCE OF PARABOLIC SURFACE REPLICATORS?

Guenter von Kiedrowski, Ruhr-University at Bochum, Germany

The first generation of chemical self-replicating systems was based on the autocatalytic reaction sequence A + B + C ⇔ ABC → C_2 ⇔ 2 C, where C is a self-complementary template molecule, A and B its precursors, ABC a termolecular complex composed of the latter constituents, and C_2 a template duplex. Several examples of such minimal replicators were reported ranging from oligonucleotides over artificial molecules to even peptides as templates. A common problem of these minireplicators is product inhibition (formation of C_2), caused by entropic stabilization of C_2 and leading to parabolic instead of exponential autocatalysis. More recent implementation of self-replicating systems utilize the cross-catalytic reaction sequences: A + A + X ⇔ AAX → XY ⇔ X + Y and B + B + Y ⇔ BBY → XY ⇔ X + Y, where X and Y are complementary templates, AAX and BBY the respective termolecular complexes, and XY the complex between templates. Again, product inhibition (formation of XY) causes parabolic growth of the replicator ensemble. Other implementations are based on collectively closed autocatalytic sets (Kauffman sets) which can be understood as multiple cross-catalytic systems. Needless to say, that product inhibition and parabolic growth is an obstacle for both, minimal and complex systems.

Exponential growth has been considered as *the* prerequisite for Darwinian evolution of replicators. It was recently demonstrated using a stepwise replication scheme called SPREAD (*s*urface-*p*romoted *r*eplication and *e*xponential *a*mplification of *D*NA analogues). Current efforts in our laboratory seek to realize an autonomous variant of the procedure. The quest was triggered by the surprizing outcome from dynamic simulations, which I will present in my lecture.

Luther, A.; Brandsch, R.; von Kiedrowski, G.: 1998, *Nature* **396**, 245-248.

i3.2

CREATION AND EVOLUTION OF NEW RIBOZYMES

Erik Schultes, Peter J. Unrau, Wendy Johnston, and David P. Bartel
Whitehead Institute for Biomedical Research and Department of Biology, MIT, 9 Cambridge Center, Cambridge MA, 02142

We have been isolating new RNA catalysts (ribozymes) from large pools of random RNA sequences in an effort to better understand the basic properties of RNA as a catalyst and to see whether these properties are compatible with the RNA world scenario. The hypothesis that certain RNA molecules may be able to catalyze RNA replication is central to the RNA world scenario. In support of this idea, we have generated an RNA that synthesizes short segments of RNA using the same reaction as that employed by protein enzymes that catalyze RNA polymerization. This ribozyme is serving as a starting point in efforts to evolve ribozymes capable of more extensive and accurate polymerization. Another challenge for the RNA world hypothesis is the source of nucleotide substrates needed for RNA polymerization. Such nucleotides must have been synthesized from simpler precursors. We have isolated RNA molecules that catalyze the synthesis of a pyrimidine nucleotide at their 3′ terminus. Optimization of these ribozymes to the point where they synthesize non-tethered nucleotides would further support the idea of an RNA world that included nucleotide synthesis and other metabolic pathways mediated by ribozymes.

Other experiments are exploring the distribution of catalysts in RNA sequence space. Examination of natural ribozyme isolates shows that the same ribozyme motif can be represented by very different sequences. Because the different sequences have descended from a common ancestor, there are likely to be neutral paths in sequence space that connect these different sequences, allowing evolutionary drift from one sequence to the other without loss of function. The set of all possible neutral paths for a particular ribozyme is thought of as a neutral network. We have evidence that neutral networks for completely different ribozyme motifs can intersect. That is, we have generated an RNA sequence that can fold into two different ribozyme motifs. Because the molecule sometimes folds into one motif and sometimes folds into the other motif, the same sequence catalyzes two different reactions. The fact that networks for functional RNAs intersect makes RNA an attractive biopolymer substrate for the birth of new function early in the evolution of life. In principle, only one ribozyme needed to emerge from arbitrary sequences at the beginning of the RNA world; all other ribozymes could have descended from this founding ribozyme. A related implication is that RNAs in modern biology with no structural or functional similarities may have common ancestry. Furthermore, our results show that evolutionary divergence can precede duplication, which differs from the canonical view of divergence following duplication.

i3.3

FROM PEPTIDE REPLICATORS TO SELF-ORGANIZED NETWORKS

M. Reza Ghadiri, Departments of Chemistry and Molecular Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037.

What are the fundamental properties that distinguish the chemistry of living systems, which gives rise to animate characteristics, from inanimate in vitro chemical transformations? In a living system, the complex blend of nonlinear molecular information-transfer processes is thought to bring about a coherent self-organized chemical system that can display emergent properties. In order to understand and ultimately mimic the properties of living systems, we feel it is necessary to begin defining the basic forms of self-organized autocatalytic chemical networks, how they can be constructed, and how the interplay of information and nonlinear catalysis can lead to the expression of emergent properties. In this lecture, within the context of de novo designed catalytic and autocatalytic peptides, I will discuss the construction of simple self-organized autocatalytic networks that begin to display some of the most basic properties of living molecular systems such as selection, adaptation, and the acquisition of new functions.

1. Lee, D. H.; Granja, J. R.; Martinez, J. A.; Severin, K.; Ghadiri, M. R. "A Self-Replicating Peptide". *Nature* **1996**, *382*, 525-528.

2. Severin, K.; Lee, D.; Martinez, J. A.; Ghadiri, M. R. "Peptide Self-Replication via Template-Directed Fragment Condensation" *Chem. Eur. J.* **1997**, *3*, 1017-1024.

3. Severin, K.; Lee, D. H.; Kennan, A. J.; Ghadiri, M. R. "A Synthetic Peptide Ligase", *Nature* **1997**, *389*, 706-709.

4. Lee, D. H.; Severin, K.; Yokobayashi, Y.; Ghadiri, M. R. "Emergence of Symbiosis in Peptide Self-Replication Through a Hypercyclic Network", *Nature* **1997**, *390*, 591.

5. Lee, D. H.; Severin, K.; Ghadiri, M. R. "Autocatalytic Networks: The Transition From Molecular Self-Replication to Ecosystems", *Curr. Op. Chem. Biol.* **1997**, *1*, 491-496.

6. Severin, K.; Lee, D. H.; Martinez, J. A.; Vieth, M.; Ghadiri, M. R. "Dynamic Error-Correction in Autocatalytic Peptide Networks", *Angew. Chem. Int. Ed.* **1998**, *37*, 126-128.

c3.4

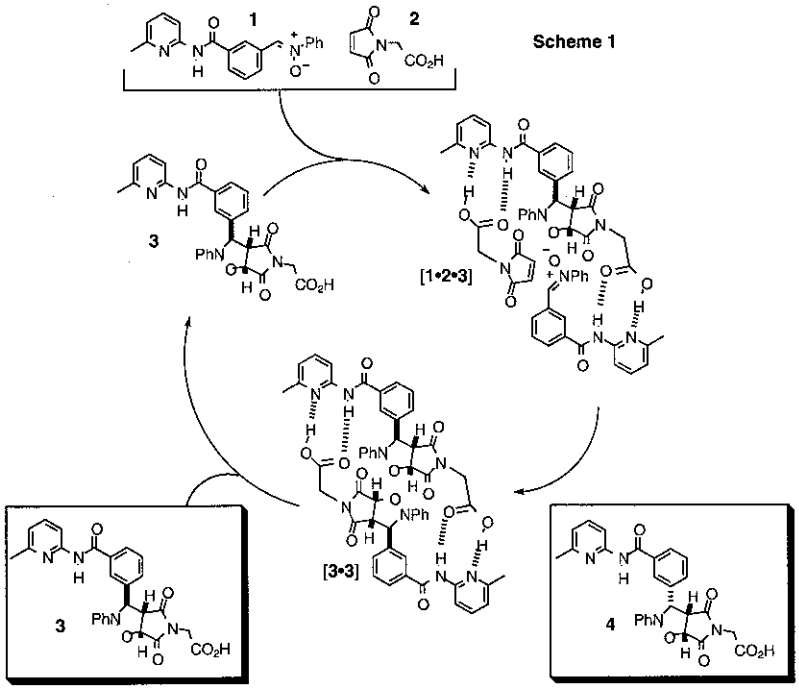
Replication and Recombination in Minimal Synthetic Systems

Victoria C Allen, Raphael M. Bennes, Douglas Philp*
and Andrew J Sinclair

School of Chemistry, University of Birmingham, Edgbaston,
Birmingham B15 2TT, United Kingdom

Examples of chemical systems capable of templating and catalysing their own synthesis – self-replicating systems – have begun to appear in the chemical literature over the last 10 years. Almost all of the successful systems have relied on the application of naturally-occurring recognition motifs based on either peptides or nucleic acids. Additionally, almost all of the well-characterised systems published to date have relied on chemical reactions such as the formation of amides and phosphate esters to construct the template. This renders the system trivial in an informational sense as the formation of the covalent bond between the replicator fragments and, hence, the template can have only one possible outcome – that is, mutation mediated by the covalent bond forming step is not possible in such systems.

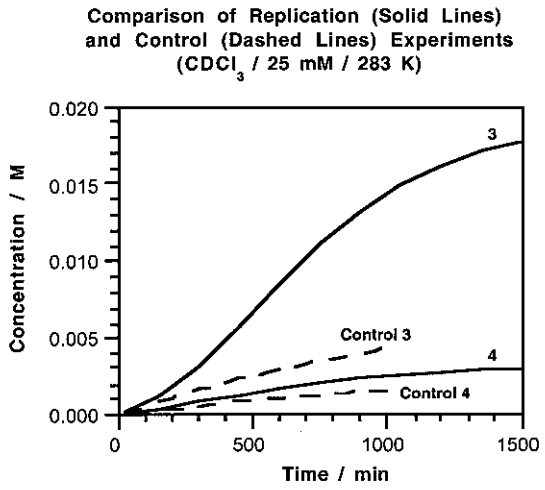
Recently, we have designed and synthesised a system (**Scheme 1**) which exploits the non-linear kinetic behaviour of replicating systems to achieve the amplification of relative stereochemistry through a replication process.



c3.4 continued

Cycloadduct **3** (**Scheme 2**) possesses the capability to replicate itself through the type of autocatalytic cycle shown in **Scheme 1** , however, its diastereoisomer **4** is incapable of templating its own formation. Therefore, when the nitrone **1** and maleimide **2** are allowed to react in $CDCl_3$ at 10°C, both stereoisomers are formed initially. However, when the concentration of **3** rises to a sufficient level, the autocatalytic cycle amplifies the formation of **3**, but not the formation of **4**. This amplification process is responsible for the sigmoidal profile (**Scheme 2**) of the concentration-time curve for the formation of **3**. Importantly, there is no crosscatalysis in this system. In other words, in addition to **4** being incapable of catalysing its own formation, it is also incapable of catalysing the formation of **3**.

Scheme 2



Additionally, the kinetics of this replicating system do not exhibit a square root relationship to the concentration of template, but rather displays a direct response to the template concentration. Therefore, one might view the outcome of this process in Darwinian terms. Cycloadduct **3** is better adapted for replication and so is able to compete more effectively for the common building blocks **1** and **2** than cycloadduct **4**. This fitness for replication results in an enhancement of the population of **3** relative to that of **4** (solid curves in the graph in **Scheme 2**) – in the absence of replication, **3** and **4** would be formed in an approximately 2:1 ratio (dashed curves in the graph in **Scheme 2**).

This presentation will discuss the experiments required to elucidate the mechanism of replication in this system – including 1H NMR studies and X-ray crystallography. Recombination experiments in which a number of replicator components compete for a common building blocks will also be discussed. The results obtained in this study will be contrasted with preliminary data obtained on two other related systems

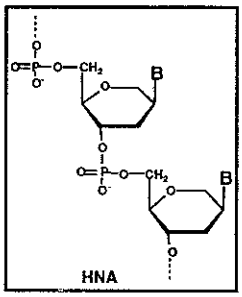
c3.5

STUDY OF NON-ENZYMATIC SYNTHESES OF RNA AND DNA ON RNA, DNA AND HNA TEMPLATES

Igor A. Kozlov, Panagiotis K. Politis, Stefan Pitsch[#], Bart De Bouvere[*], Roger Busson[*], Arthur Van Aerschot[*], Piet Herdewijn[*]& Leslie E. Orgel

The Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA, 92186; [#]Swiss Federal Institute of Technology, Universitatstrasse 16, CH-8092, Zurich, Switzerland and [*]Laboratory of Medical Chemistry, Rega Institute, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Non-enzymatic oligomerization reactions of activated nucleotide 5'-phosphates on RNA, DNA and hexitol nucleic acid (HNA) templates are compared with respect to oligomerization efficiency, information transfer and enantiomeric cross-inhibition.



The ability of decacytidylate oligomers to facilitate oligomerization of the 5'-phosphoro-2-methylimidazolides of guanosine and the ability of heterosequence oligomers to transfer information decreasing in the order HNA>RNA>DNA. Strong enantiomeric cross-inhibition is observed on RNA and DNA decacytidylate templates but the inhibition is much less severe on an HNA decacytidylate template. Comparison of the oligomerization efficiencies of the activated of guanosine and deoxyguanosine on decacytidylate HNA, RNA and DNA templates under various conditions suggests strongly that only nucleic acid double helices with the A-structure support efficient template-directed synthesis.

Kozlov, I. A., Politis, P. K., Pitsch, S., Herdewijn, P., Orgel, L. E. : 1999, J. Am. Chem. Soc., **121**, 1108-1109.

Kozlov, I. A., Politis, P. K., Van Aerschot, A., Busson, R., Herdewijn, P., Orgel, L. E. : 1999, J. Am. Chem. Soc., *in press*.

c3.6

TOWARDS *IN VITRO* SELECTION OF RIBOZYMES WITH POLYMERASE ACTIVITY

Kourosh Salehi-Ashtiani and Jack W. Szostak
Harvard Medical School Department of Genetics, and Massachusetts General Hospital Department of Molecular Biology, Boston, MA 02114

Isolation of ribozymes with true polymerase activity remains an essential goal for establishing the plausibility of the "RNA World" hypothesis. We have designed an *in vitro* selection strategy for the isolation of ribozymes with template directed DNA polymerase activity for incorporation of natural nucleoside triphosphates. Once isolated, these ribozymes are then to be further evolved to perform RNA dependent RNA polymerization and other related functions. This approach (*i.e.*, selection for DNA polymerase activity) gives the advantage of directly selecting for polymerization rather than a related function and thus increasing the likelihood of isolating the desired catalysts. We have based our selection on discrimination mediated by restriction endonucleases, where primers that become extended by a single or multiple nucleotides are selected and distinguished from non-extended ones. In reconstitution experiments, we have found an enrichment factor of ~50 fold per selection cycle for molecules that have an additional base. With multiple rounds of selection, this level of enrichment will be sufficient to isolate active polymerases if they are present in the pool.

An important criteria for assessing the feasibility of this selection was to determine the background rate for incorporation of a single unmodified deoxynucleotide in our experimental setting. We therefore synthesized a deoxyoligonucleotide that forms a hairpin with a protruding 5' end, leaving a 3' terminus that can be extended over a templated G. Upon incubation of this oligonucleotide with [α^{32}P]dCTP, we were able to detect labeling of the oligonucleotide. Further analysis of the labeled product indicated that part of the labeling is template dependent and is limited to the 3' of the molecule, while a portion of the labeling is not template dependent and can occur to the 5' half. Based on our preliminary measurements of the templated reaction, the rate acceleration needed for selection of a polymerase is within the range of enhancements typically achieved by ribozymes selected for various other functions. We are currently carrying out the selection.

c3.7

IN VITRO EVOLUTION OF LIGASE RIBOZYMES: TOWARDS AN RNA-DEPENDENT RNA POLYMERASE

Kathleen E. McGinness and Gerald F. Joyce
The Scripps Research Institute, La Jolla, CA 92037

One of the central concepts in the RNA World hypothesis is that of a self-replicating RNA molecule. Such a molecule would be required to possess the ability to catalyze the polymerization of mononucleotides in a template-directed fashion, with high fidelity. We have developed an *in vitro* evolution approach to select for RNA molecules that are capable of this polymerase activity.

The scheme relies on the evolution of ligase ribozymes in a continuous fashion. Continuous evolution allows for the concomitant selection and amplification of ribozymes in a single reaction vessel, with amplification being dependent on ribozyme catalysis (Wright & Joyce, 1997). In this format, a pool of ligase ribozymes are challenged to bind a DNA-RNA chimeric substrate and catalyze three successive phosphoester transfer reactions, completing one strand of the T7 RNA polymerase promoter element. Complementary cDNA strands of reacted RNA molecules are then formed by reverse transcription, completing the double-stranded T7 RNA polymerase promoter element. Subsequent forward transcription by T7 RNA polymerase produces progeny RNA molecules that are eligible to proceed though the next cycle.

Employing the continuous evolution system, we have been able to evolve ribozymes that are capable of catalyzing at least three successive phosphoester transfer reactions—two mononucleotide additions and one ligation. This extends previous studies concerning polymerase-like activity in ribozyme catalysis (Been & Cech, 1988; Ekland & Bartel, 1996).

Been, M.D. and Cech, T.R. : 1988, *Science* **239**, 1412.
Ekland, E.H. and Bartel, D.P. : 1996, *Nature* **382**, 373.
Wright, M.C. and Joyce, G.F. : 1997, *Science* **276**, 614.

cA3.1

DIRECT OBSERVATION OF THE SELF ASSEMBLY OF POTENTIALLY PREBIOTIC PURINE MOLECULES ON MINERAL SURFACES BY SCANNING TUNNELING MICROSCOPY

Wolfgang M. Heckl, Ludwig-Maximilians-Universität München, Institut for Cristallography und Applied Mineralogy and Stephen J. Sowerby, Dept. of Biochemistry, University of Otago Dunedin, New Zealand

The formation of monolayers of the purine and pyrimidine bases through physisorption mediated molecular self-assembly at the solid-liquid interface, has been proposed to have a functional role in the emergence of terrestrial life (1,2,3). The application of near field microscopy techniques, namely scanning tunneling microscopy (STM), to these supramolecular surface structures has allowed real space analysis with atomic scale lateral resolution. We have suggested that purine and pyrimidine monolayers could be candidates for a stationary phase in organic molecule separation systems, and as templates for the assembly of higher ordered polymers at the prebiotic solid-liquid interface. We will present the concept of organic monolayer structure determination by combining real space STM technique, low energy electron diffraction and molecular modelling (4, 5) which has been applied to all DNA bases and the RNA base uracil on various inorganic mineral surfaces. In some cases, like for adenine, the spontaneous molecular self assembly from liquid solution leads to localized chiral symmetry break which may have some role in the origin of biomolecular optical asymmetry. The possiblity that purine-pyrimidine arrays assembled on naturally occuring mineral surfaces might act as templates for biomolecular assembly of amino acids is discussed.

Lit.:

1. The Role of Self assembled purine and pyrimidine bases in the emergence of life, S. J. Sowerby and Wolfgang M. Heckl , Origin of Life and Evolution of the Biosphere, 28, (1998) 283-310
2. Chiral Symmetry Breaking During the Self-assembly of Monolayers from Achiral Purine Molecules, S. J. Sowerby and Wolfgang M. Heckl and G. B.Petersen, J. Mol. Evol., 43. 419-424 (1996)
3. Self-assembly at the Prebiotic Solid-liquid Interface: Structures of Self-assembled Monolayers of Adenine and Guanine Bases Formed on Inorganic Surfaces, S.J. Sowerby, M. Edelwirth and W.M. Heckl, J.Phys.Chem., 102(30), 5914-5922, (1998)

cA3.1 continued

4. Molecular Mechanics Simulation of Uracil Adlayers on Molybdenum Disulfide and Graphite Surfaces, S.J. Sowerby, M. Edelwirth and W.M. Heckl, Appl. Phys. Lett. A66, S649-653(1998)
5. Molecular mechanics study of hydrogen bonded self-assembled adenine monolayers on graphite, M. Edelwirth, J. Freund, S. J. Sowerby and W. M. Heckl, Surface Science 417,, 201-209, (1998)

cA3.2

SELF-ASSEMBLED TWO-DIMENSIONAL GENETIC SYSTEMS

Stephen J. Sowerby[1], Peter A. Stockwell[1], Nikola Kasabov[2] and George B. Petersen[1].

[1]Department of Biochemistry, [2]Department of Information Science, University of Otago, P.O. Box 56, Dunedin 9001, New Zealand.

It is generally believed that the precursors of modern cells arose on the surface of the primitive earth and it is clear that a key step in the origin of life was the development of a primitive, informational, self-replicating system. Acceptance that such a system arose *ab initio* implies that the conditions required for its initial development can be found by experiment. A plausible starting point comes from the demonstration that purine and pyrimidine bases of DNA can self-assemble as two-dimensional monolayers on the surfaces of crystalline solids. Such self-assembly is *spontaneous* and in view of the putative prebiotic availability of this class of compounds, suggests that monolayers could have had prebiotic relevance. In such monolayers, the bases are *fixed relative to one another* on an inorganic surface and the structure is stabilised by intermolecular hydrogen bonds. Experiments have shown that monolayers of mixed composition form aperiodic quasicrystalline structures which suggests that they could act as a primitive form of information storage. We believe that these systems could be relevant to the evolution of biological informational systems because in modern biochemistry, the information is, in essence, the order of the purine and pyrimidine bases which are *fixed relative to one another* by attachment to the backbone of DNA and it is extremely unlikely that DNA-like molecules were present in the prebiotic milieu. It has been proposed that the coding capabilities of these matrices could be implemented through a scaffolding mechanism whereby specific amino acids physicochemically interact with different dimeric combinations of bases such that the amino acids are stereochemically positioned for subsequent peptide bond formation. The formation of template encoded catalytic peptides would offer the possibility of informational self-replication that resembles elements of modern biochemistry. Using this paradigm we have undertaken experimental studies on these two-dimensional chemical systems and have applied computer simulation to investigate two-dimensional virtual chemistries.

Sowerby, S.J. and Heckl, W.M.: 1998, *Origins of Life Evol. Biosph.* 28, 283.
Sowerby, S.J., Heckl, W.M. and Petersen, G.B.: 1996, *J. Mol. Evol.* 43, 419.

cA3.3

THE OLIGONUCLEOTIDES SYNTHESIS FROM NON-ACTIVATED MONONUCLEOTIDES, DIRECTED BY POLYNUCLEOTIDE TEMPLATE ADSORBED ON THE MINERAL SURFACE

Vladimir A. Otroshchenko and Nelly V. Vasilyeva
A.N. Bach Instiutute of Biochemistry, Russian Academy of Sciences, Moscow, Russia. E-mail <inbio@glas.apc.org>

The abiotic formation of oligo- and polynucleotides, in particular, their non-enzymatic template-directed synthesis, is of principal importance for the development of primitive organisms. In the most attempts to mimic the abiotic template-directed oligo- and polynucleotides synthesis, the chemically-activated mononucleotides were used as the substrate material. All the success in the water-phase nucleotide condensation has been connected with the using of chemically activated purine nucleotide derivatives (mainly, guanosine-5'-phospho-2-methylimidazolides) which condensation was directed by the pyrimidine-rich, especially cytosine-rich, templates. The attempts to demonstrate the pyrimidine nucleotide condensation were unsuccessful, and a view has been expressed, that such a synthesis cannot be achieved in chemical models where the non-activated pyrimidine nucleotide precursors interacted with a GC-free template [Hill, *et al.*, 1993].

To demonstrate the abiotic template-directed nucleotide condensation, in this work we used the 5'-nucleoside monophosphates adsorbed from a water solution by a polynucleotide template, pre-adsorbed on the clay mineral surface. This model is in a agreement with the natural processes, especially considering the success in the ribose-2,4-diphosphate formation from the prebiotically relevant reaction of glyceraldehyde monophosphate and formaldehyde [Orgel, 1998].

When the [^{14}C] 5'-UMP adsorption by the polyA template pre-adsorbed, in its turn, on the montmorillonite surface was followed with a heating up to 80°C, it resulted in the oligo-U synthesis (at least, up to the U_5) and in the formation of a canonic polyA–polyU complex. The RNAse-A treatment of polyU fraction resulted in a complete degradation of the oligonucleotide to mononucleotides. In case of the substitution of a single UMP substrate with the equimolar 5'-nucleoside monophosphates mixture, the oligomer formed consisted of UMP by more than 70%, thus pointing to a template-directed specificity of the reaction. The above-mentioned results pointed to the non-enzymatic and template-directed synthesis of pyrimidine oligonucleotides from the non-activated nucleoside monophosphate molecules interacting with purine template adsorbed on mineral surface.

Hill, A.R., Jr., Orgel, L.E., and Wu, T.: 1993, Origins of Life **23**, 285-290.
Kawamura, K. and Ferris, J.P.: 1994, JACS **116**, 7564-7572.
Orgel, L.E.: 1998, TIBS **23**, 491-495.

cA3.4
A COVALENTLY-LINKED BASE PAIR

Kui Gao & Leslie E. Orgel*
The Salk Institute for Biological Studies,
10010 North Torrey Pines Road, La Jolla, California 92037, USA

The normal base pairs in nucleic acid duplexes are held together by hydrogen bonds. We used molecular modeling techniques to design a covalently-linked base pair which can replace a normal base pair without distorting the structure of the standard double helix. We inserted this base pair into a precursor of a DNA double helix by chemical synthesis and demonstrated efficient non-enzymatic template-directed ligation between the covalent base pair and a standard oligonucleotide. This shows that the covalently-linked base pair can incorporate into a nucleic acid double helix structure very well, and thus covalently link the two strands. Our work suggests that it may be possible to base a replication system on recognition by covalent bonding. This novel approach to the construction of an analogue of a nucleic acid duplex may have applications to biochemistry and biotechnology.

cA3.5

NON-ENZYMATIC TEMPLATE-DIRECTED LIGATION OF 2'-5' OLIGORIBONUCLEOTIDES

Hiroaki Sawai, Makoto Wada, 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 early stage of the origins of life. 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 The 3'-5' linked poly- or oligoribonucleotides have been proved to serve as a template in the nonenzymatic oligomerization or ligation of 3'-5' oligoribonucleotides. Recently, Ferris et al. and Prakash et al. demonstrated the template-directed synthesis of oligo(G)s from the activated guanylate on 2'-5' linked oligo(C) templates. However, template-directed ligation of 2'-5' linked short-chained oligoribonucleotides on the complementary 2'-5'- or 3'-5'-linked oligoribonucleotides, which could take place in the prebiotic chemical process, has been a matter of speculation..

Thus, we investigated nonenzymatic template-directed ligation of 2'-5' linked short-chained oligoribonucleotides on 2'-5' or 3'-5' linked oligoribonucleotides in two systems; (1) ligation of 2'-5' linked diadenylate on 2'-5' or 3'-5' dacauridylate template, and (2) ligation of mixed sequence of 2'-5' linked tetramer (pACUG) on 2'-5' linked complementary oligoribonucleotide templates. The 2'-5' linked homo-oligonucleotides were obtained by model process of prebiotic synthesis of oligoribonucleotides using uranyl ion catalyst in aqueous solution . We found that decauridylate containing exclusively a 2'-5' phosphodiester bond ([2'-5']U_{10}) served as a template for the synthesis of oligoadenylates [oligo(A)s] from the 5'-phosphorimidazolide of 2'-5' diadenylate (ImpA2'p5'A). Joining of [2'-5']U_{10} and ImpA2'p5'A also took place in substantial amounts to yield long-chained oligoribonucleotides in the template-directed reaction. The 3'-5' linked decauridyalte ([3'-5']U_{10}) also promoted the template-directed synthesis of oligo(A)s from ImpA2'p5'A but in lower efficiency compared with [2'-5']U_{10}. The mixed sequence of 2'-5' linked tetramer also condensed each other on a complementary 2'-5' linked oligoribonucletides forming the corresponding octamer. The results indicate that short-chained RNA oligomers with a 2'-5' phosphodiester bond produced in the prebiotic process form a helix between the complementary strands and could lead to longer oligoribonucleotides by template-directed chain elongation.

cA3.6

THE PREBIOTIC SYNTHESIS OF THE COMPONENTS OF PEPTIDE NUCLEIC ACID, A POSSIBLE FIRST GENETIC MATERIAL

Kevin E. Nelson, Matthew Levy, and Stanley L. Miller

Department of Chemistry and Biochemistry
University of California, San Diego
La Jolla, California 92093-0506, USA

The numerous problems with synthesizing RNA from ribonucleotides and with synthesizing the ribonucleotides themselves raise serious questions about whether RNA was the first genetic material. An attractive alternative is peptide nucleic acid (PNA), nucleotide analogs using a polypeptide backbone of 2-aminoethyl glycine (AEG) with the nucleobases attached through an acetic acid (Nielsen, P. E., *et al.*, 1991). PNA has been proposed as a primitive genetic material (Nielsen, P. E., 1993 and Miller, S. L., 1997). We show here that the monomeric components of PNA are prebiotic compounds. This makes a plausible case for PNA as the first genetic material.

AEG is produced in low yields in both the spark discharge reaction and in the polymerization of NH_4CN. Ethylenediamine is also produced from these reactions. Ethylenediamine has also been reported as major product of the photolysis of CH_4 and NH_3 in the presence of water (Ogura, K., *et al.*, 1988). A likely route to AEG from ethylenediamine is via the Strecker synthesis which we have found produces AEG in high yield (11-78%) and at high dilution (10^{-5}M). The modified purine bases are obtained from the polymerization of NH_4CN in the presence of glycine. The modified pyrimidines are synthesized efficiently from the reaction of the prebiotic compounds cyanoacetaldehyde and hydantoic acid.

These results show that AEG and the N-acetic acid modified bases may have been present on the primitive earth and under favorable conditions would have been abundant. The high solubility of AEG and its lactam monoketopiperazine suggest that these compounds might concentrate in drying lagoons to form polymers. Preliminary experiments indicate the AEG polymerizes readily to give the PNA backbone. This is the first example of a plausible prebiotic genetic material. However, this does not exclude other possibilities.

Miller, S. L.: 1997, Nature Struct. Biol. **4**, 3.
Nielsen, P. E., Egholm, M., Berg, R. H., Buchardt, O.: 1991, Science **254**, 1497.
Nielsen, P. E.: 1993, Origins of Life Evol. Bioshpere **23**, 323.
Ogura, K., Migita, C. T., and Yamada, T.: 1988, Chem. Lett., 1563.

cA3.7

WHY NATURE CHOSE α-AMINO ACIDS —Natural Selection and Chemical Selectivity

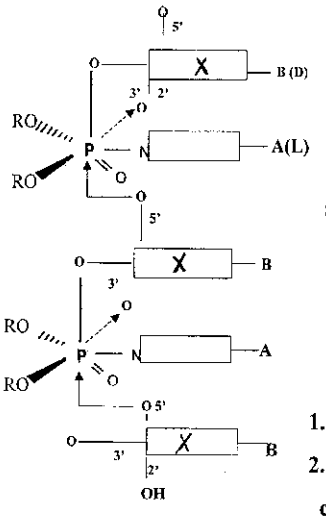
Yu-Fen Zhao, Pei-Sheng Cao
Bioorganic Phosphorus Chemistry Laboratory
Department of Chemistry School of Life Science and Engineering
Tsinhua University, Beijing, China 100084

Protein of all species, from bacteria to humans, are made of the same set of twenty standard amino acids. Nineteen of them are α-amino acids with a primary amino group and a carboxylic group attached to a central carbon atom. Why nature chose α-amino acids for the protein backbone?
Is there any connection between the natural selection and the chemical selectivity among all different amino acids? For example, N-(O, O-diisopropyl)-phosphoryl-α-alanine proceeded the mono- and di- esters exchange if the reaction medium contained the hydroxyl compounds. But under the same conditions, the β- alanine derivative, a chemical inert species, did not react with any hydroxyl compounds.

In the literature, Fox[1] also had showed that by treatment with polyphosphoric acid at 273K the nucleosides but not the deoxynucleosides could be converted into the nucleotides. Schwartz isolated the 2', 3' and 5'-AMP by mixing the adenosine with linear polyphosphoric acid[2]. These nucleoside synthesis were independent on the peptide synthesis. In our laboratory, it was found that N-phospho-α-amino acids not only could self-assemble into oligopeptides, but also could phosphorylate the nucleosides or the deoxynucleosides to give mononucleotide, dinuleotides and their aminoacyl-conjugate. The P31-NMR HPLC, FAB-MS, CE(Capillary Electrophoresis), CE-MS were used for multiple checks on the products. Hence, the N-phospho-α-amino acid and the co-evolution of protein and nucleic acids were proposed.[3,4,5] However, from the reaction of N-phospho-β-amino acids, neither the peptide nor the nucleotide was detected. This chemical selectivity might provide the clues for why nature chose α-amino acids as the protein building blocks.

cA3.7 continued

Assembly of Nucleoside by Phospho-amino acids



5'XpXpXpXp3' + aa

5' ⟶ 3'

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References

1. Fox, S.W. and Harada, K. *Arch.*: 1960, *Biochem. Biophys.* **86**, 281.
2. Schwartz, A.: 1968, *Nature*, **218**, 443.
3. Zhou, W., Ju, H., Y. and Zhao, Y.F., Wang, Q.G. and Luo, G.A.: 1996 *Origins of Life and Evolution of the Biosphere,* 279-285
4. Zhao, Y.F. and Cao, P.S.: 1994, *J. of Biological Physics,* **20**, 283.
5. Zhao, Y.F. and Cao, P.S.: 1996, *"Chemical Evolutionl Physics of the Origin and Evolution of Life"*, 279-285. Chela-flores and F.Raulin (eds).

cA3.8

THE PHOTOCHEMICAL PROPERTIES OF PTERINS RELEVANT TO FUNCTION IN EARLY EVOLUTION

Mikhail S. Kritsky, Taisiya A. Telegina, Tamara A. Lyudnikova, Michael P. Kolesnikov, Anna V. Umrikhina. Yulia L. Vechtomova, and Eugeniy A. Mironov*.
A.N. Bach Institute of Biochemistry and
*A.N. Nesmeyanov Institute of Organoelement Compounds,
Russian Academy of Sciences,
Moscow, Russia. E-mail <inbio@glas.apc.org>

The widespread photoreceptor function in organisms of the pteridine derivatives, pterins, as well as of isoalloxazine derivatives, flavins, correlates with these compounds availability from prebiotic processes, in particular, from the amino acids thermolysis. The pterins and flavins yield in such reactions was found to increase after the initial amino acid ratio had been optimized up to *glu:gly:lys = 8:3:1* (the presence of glycine was absolutely essential). In solid thermolytic product pteridines were detected by laser-excited fluorescence spectroscopy. After their purification by a combination of GF with IEC, AC and TLC, the products were identified by their fluorescence emission and excitation spectra in solution with 6- and 7-substituted pterins: leucopterin, xanthopterin and *iso*-xanthopterin.

The photoexcited (triplet) pterins molecules in solution were shown to oxidize under anaerobic conditions the high potential electron donors to produce the reduced pterins. The partially reduced dihydropterins were as well capable to a further photochemical reduction to tetrahydroforms. The ethylene diamine derivatives (EDMA, EDDA and EDTA), histidine and tyrosine acted as good donors for such reactions. The heterocycle side groups strongly influenced the reduction rate, i.e. the 7-substituted pterins showed a diminished capacity to photoreduction (up to its full loss) as compared to the non-substituted molecules. The EPR spectroscopy revealed photogeneration of the pterins free radicals in the presence of electron donors. The minimal temperature at which the 7-substituted and the non-substituted pterins radicals could be detected, correlated with the photoreduction rate of these compounds, thus suggesting the role of pterin radicals in this reaction.

Pterins, including those 7-substituted molecules which do not produce reduced forms after irradiation, photocatalyzed the electron transfer from donor (*i.e.* EDTA) to acceptor (cytochrome *c*). The latter reaction proceeded both in the presence and absence of O_2, showing a significantly different kinetics under the aerobic and anaerobic conditions. The results will be discussed in context of evolution of the photobiological processes.

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cB3.1
RNA-CATALYZED PYRIMIDINE SYNTHESIS

Peter J. Unrau and David P. Bartel
Whitehead Institute, 9 Cambridge Center, Cambridge MA, USA 02142

Prebiotic pyrimidine synthesis has not been demonstrated and is likely to have been difficult (Orgel & Lohrmann). Therefore, the synthesis of nucleotides by RNA catalysts (ribozymes) would have been essential to sustain an RNA world. Modern metabolism synthesizes pyrimidines using 5'-phosphoribosyl 1'-pyrophosphate (pRpp) together with orotate or uracil. The chemistry of this reaction, nucleophylic attack on carbon following release of pyrophosphate, is predominantly SN_1 in character (Tao *et al.*), which differs from known RNA catalyzed reactions. The small size of the pyrimidine base also poses interesting questions as to the ability of RNA catalysts to recognize metabolic substrates.

We have selected from random sequence three families of ribozymes able to promote pyrimidine nucleotide synthesis (Unrau & Bartel). Prior to selection, the RNA pool ($>10^{15}$ different sequences, having 228 random positions) was linked to pRpp and then incubated with 4-thiouracil. Ribozymes able to synthesize 4-thiouridine where selected using thiophylic reagents and enriched by 11 rounds of *in vitro* selection. Nucleotide synthesis by these ribozymes was at least 10^7 times faster than the uncatalyzed rate of 4-thiouridine synthesis. The ribozymes displayed a high specificity for 4-thiouracil, working 10^4 times slower with uracil.

One of the ribozymes was subjected to mutagenesis and additional rounds of selection to isolate faster variants and determine its secondary structure. Some new isolates have rates approaching 100 $M^{-1}min^{-1}$ (>20 times faster than the parent sequence). Structurally, the ribozyme is one of the largest known, with 62-76 nucleotides involved in base pairing. We are currently optimizing the ribozyme sequence with the goal of investigating the synthesis of free nucleotides using pRpp and 4-thiouracil.

Orgel, L.E. & Lohrmann, R.: 1974 Acc. Chem. Research **7** 368-377.
Tao, W., Grubmeyer, C. & Blanchard, J.S.: 1996 Biochemistry **35** 14-21.
Unrau, P.J. & Bartel, D.P.: 1998 Nature **395** 260-263.

cB3.2

SELECTION OF CATALYTIC RNA WITH REDUCED DIVALENT CATION DEPENDENCE BY CONTINUOUS EVOLUTION *IN VITRO*

Niles Lehman
Dept. of Biological Sciences, Univ. at Albany, SUNY, Albany, NY 12222

Most catalytic RNA's are metalloenzymes, that is, they depend on metal cations for proper folding and/or catalysis. For example, the naturally-occurring form of the *Tetrahymena* self-splicing rRNA IVS has an absolute dependence on Mg^{2+} or Mn^{2+} for catalysis, although a portion of this cationic requirement for folding into a proper 3-dimensional conformation can be alleviated with other divalent metal ions. Recently though, DNAzymes have been selected from completely random pools that show little, if any, dependence on divalent cations (Geyer and Sen, 1997).

The goal of the current study was to explore the possibility that a ribozyme that has a strong Mg^{2+}-dependence for catalytic activity could be "weaned" of its Mg^{2+} requirement by *in vitro* selection. We utilized the continuous evolution protocol developed by Wright and Joyce (1997) to select variants of a ligase ribozyme that were proficient catalysts at low Mg^{2+} concentrations. Using a "slow wean" strategy of dropping the Mg^{2+} concentration by 2.5 mM every 3 rounds of continuous evolution, an initially heterogeneous population of RNA sequences was focused to contain a single family of sequences that was active in less than 4 mM free Mg^{2+}. Each member of this family had a consensus sequence containing 9 point mutations from the starting sequence on which the initial randomized pool was based. The consensus sequence is indeed more active than the starting sequence; a standard assay of ligation rate using excess substrate revealed that 40% of the selected sequence can perform ligation in a 10-second reaction, compared to less than 24% using the starting sequence.

Kinetic analysis of both the starting and selected RNA sequences revealed an interesting phenomenon: even in vast substrate excess neither sequence can perform 100% ligation under optimal conditions (25 mM Mg^{2+}, 24-hour incubation at 25°C). Thus the possibility existed that preparations of such RNA, though homogeneous in 1° sequence, contained both active and inactive forms of the ligase ribozyme. High-resolution acrylamide gel electrophoresis discounted the likelihood that some sequences were lacking the 5'-triphosphate critical for phosphodiester ligation, so an alternative explanation was sought to explain partial inactivity. Native gel electrophoresis and complementary temperature-dependent kinetic analyses suggest instead that alternative 2° structures can exist in these genotypically-homogeneous populations, with ramifications for the efficiency of selection in RNA populations in the RNA World.

Geyer, C.R. and Sen, D. :1997, Chemistry and Biology **4**, 579.
Wright, M.C. and Joyce, G.F. :1997, Science **276**, 614.

cB3.3

SELECTION AND DESIGN OF ALLOSTERIC RIBOZYMES

Michael P. Robertson and Andrew D. Ellington
University of Texas at Austin, Austin TX, USA 78712

The RNA World hypothesis proposes that at some period during the evolution of life, RNA molecules performed the dual functions of genetic storage and enzymatic catalysis. The possibility that RNA can function in both these capacities was demonstrated with the discovery of catalytic RNA in the early 1980s. Since then the types of chemical reactions that RNA has been shown to catalyze has steadily increased and with it the feasibility of an all RNA metabolism. However, a key aspect of modern biocatalysis is the ability of enzymes to attenuate their level of activity in response to ever-changing cellular conditions. Presumably, a similar regulation mechanism would also be necessary to sustain a reasonably complex RNA-based metabolism.

We have isolated and designed several RNA ligase ribozymes whose activity is regulated by the presence of either oligonucleotide or small molecule effectors. An oligonucleotide-dependent ligase (L1) was isolated from a randomized pool of RNA using in vitro selection. This ribozyme's activity in the presence of a specific oligonucleotide effector is 10,000-fold greater than when the effector is absent. Other allosterically regulated ribozymes were engineered by replacing a non-essential stem loop of L1 with various small molecule binding RNA aptamers to create aptamer-ribozyme hybrids (aptazymes). Ligand binding induces a conformational change in the aptamer domain which is propagated to the ribozyme domain through a short linking stem which allows the ribozyme domain to adopt an active conformation. The aptazyme's level of response to ligand can be adjusted by altering the basal stability of the stem linking the aptamer and ribozyme domains. This approach has resulted in aptazymes that are regulated by ATP, theophylline, and FMN with activations of 800, 1600, and 250 respectively.

These results demonstrate that ribozyme activity can be regulated in much the same way that protein enzymes are regulated in contemporary biochemistry. This ability of RNA to be controlled by various potential metabolic intermediates contributes an additional layer of sophistication to ribozyme catalysis and increases the plausibility that a complex metabolism based solely on RNA could have once existed.

cB3.4

A COMPLEX RIBOZYME THAT LACKS CYTIDINE

Jeff Rogers & Gerald F. Joyce
The Scripps Research Institute, La Jolla, CA

In vitro evolution was used to isolate a complex RNA ligase ribozyme that completely lacks the nucleotide cytidine. Cytidine was selected because RNA composed of adenosine, guanosine and uridine can still form two base pairs (A-U Watson-Crick and G-U wobble pairs) and because cytosine is by far the least stable of the four ribonucleotides. The starting molecule was E100, which isolated from continuous evolution (Wright & Joyce). E100 was randomized, treated with bisulfite and transcribed in the absence of cytidine. 24 rounds of selection were done with cloning and sequencing done after every eight rounds. The resulting 155-nucleotide ribozyme, which is completely devoid of cytidine, catalyzes the template-directed ligation of an RNA substrate. All 37 cytidines that had been present in the molecule were either replaced by uridine, or a more complex arrangement of adenosine, guanosine and uridine residues. The catalytic rate of the cytidine-free ribozyme is approximately 10^5 times faster than the uncatalyzed rate of template-directed ligation. This cytidine-free ribozyme shows that it is possible to maintain both RNA structure and function in a three base genetic code.

Wright, M. C. and Joyce, G. F., *Science* **276**, 614 (1997)

cB3.5

TRANSITION FROM GENETIC CODE EVOLUTION TO THE LAST COMMON ANCESTOR

J. Tze-Fei WONG and Hong XUE
Department of Biochemistry, Hong Kong University of Science & Technology, Clear Water Bay, Hong Kong, China

Since the universal genetic code is shared by all major groups of living organisms, evidently it was acquired by the last common ancestor (LCA) of living organisms prior to their divergence. Indeed, completion of the universal code through the coevolution of genetic code and ensemble of protein amino acids could be one of the important factors that made possible the emergence of LCA (1-3). There has been considerable confusion arising from microbial genome sequencing regarding the nature of LCA (4). To delineate the transition from the coevolution of the genetic code and protein amino acids to the evolution of LCA, the encoding of Trp is of particular significance because in all likelihood Trp was one of the last amino acids to join the ensemble of encoded amino acids. Accordingly we have developed hyperexpression systems for Trp-tRNA synthetases (TrpRS) as well as $tRNA^{Trp}$ (5-7). We have now analysed the cross reactivities between TrpRS and $tRNA^{Trp}$ molecules both from all three biological kingdoms - Bacteria, Archaea and Eucarya, and their correlation to the molecular sequences, in order to determine the relationship between genetic code evolution and the evolutionary development of LCA into the three kingdoms of life.

(This study was supported by the Research Grants Council of Hong Kong.)

(1) Wong JT (1975) Proc. Nat. Acad. Sci. USA 72:1909-1912
(2) Wong JT (1976) Proc. Nat. Acad. Sci. USA 73: 2336-2340
(3) Wong JT (1988) Microbiol. Sci. 5:174-181
(4) Pennisi E (1998) Science 280:672-674
(5) Shi W, Chow KC and Wong JT (1990) Biochem. Cell Biol. 68:492-495
(6) Xue H, Shen W, Giege R and Wong JT (1993) J. Biol. Chem. 268:9316-9322
(7) Xue H, Shen W and Wong JT (1993) J. Chromat. 613:247-255

cB3.6

SELECTION OF RNA-BINDING PEPTIDES FROM RANDOM LIBRARIES

Kazuo Harada[1] and Alan D. Frankel[2]
[1]Department of Life Science, Tokyo Gakugei University, Koganei, Tokyo 184-8501, Japan
[2]Department of Biochemistry and Biophysics, University of California, San Francisco
San Francisco, CA 94143-0448, USA

The arginine-rich RNA-binding motif (ARM) is a short RNA-binding peptide motif (15-20 amino acids in length) which has been identified in viral proteins such as HIV Rev, HIV and BIV Tat, as well as a number of bacteriophage (λ, ϕ21, and P22) antiterminator N proteins. Studies of the interactions of ARMs and their RNA sites have shown that both the structure of the peptide and the RNA targets that they recognize are diverse, and that the ARM may be a particularly versatile motif. Many of the amino acid determinants of these interactions have been determined, and it appears that other than arginine, very few other amino acids (predominantly hydrophilic amino acids) are required for tight and specific binding. This low sequence complexity and versatility of the ARM lead us to hypothesize that arginine-rich RNA-binding peptides may have arisen readily from random peptide mixtures of relatively low complexity early in evolution and played a role in the transition from an RNA world to an RNA-protein world.

In order to test how readily RNA-binding arginine-rich peptides may have been selected from random peptide mixtures, we created a number of relatively small combinatorial libraries and attempted to identify peptides that bind to the HIV RRE hairpin. We used a bacterial system based on λ N antitermination, that consists of an N-expression plasmid and a reporter plasmid containing the RNA site and transcriptional termination elements upstream of LacZ, so that peptide-RNA binding and resulting antitermination can be visualized by β-galactosidase activity. We were able to select WT Rev-like peptide from one library consisting of four amino acids (R, S, N, and H), and novel peptide sequences from another library consisting of three amino acids (R, S, and G). We have also used an evolutionary approach consisting of mutagenesis and selection to identify variants of one peptide, obtained from the three amino acid RSG library, into a stronger RRE-binder. These results demonstrate the low sequence complexity needed to select specific RNA-binding peptides, as well as the ease with which RNA-binding affinity can be "evolved", therefore suggesting a possible role of arginine-rich peptides early in evolution.

cB3.7
OPTIMIZED SYNTHESIS OF RNA-PROTEIN FUSIONS FOR *IN VITRO* PROTEIN SELECTION

Rihe Liu and Jack W. Szostak
Department of Molecular Biology
Massachusetts General Hospital, Boston, MA 02114

An mRNA-protein fusion consists of a protein sequence covalently linked to the 3' end of its own mRNA via its C terminus. The fusions are generated by *in -vitro* translation of appropriate mRNA templates with puromycin attached to their 3' end via a short DNA linker. Because the coding RNA sequence and the polypeptide sequence are covalently linked as a single molecule, RNA-protein fusions provide a method for reading and amplifying a protein sequence after it has been isolated based on its function, and therefore allow the principle of *in vitro* selection to be applied to proteins. Through a combination of a flexible linker and appropriate posttranslational incubation, the efficiency of fusion formation has been increased significantly. The optimized mRNA fusion system allows protein libraries containing as many as 10^{14} members to be generated, more than any other protein selection techniques. Fusion based selections provide a powerful approach to understanding protein evolution and discovering protein interactions with protein, nucleic acid, and small molecule targets.

cB3.8

IN VITRO SELECTION OF FUNCTIONAL PROTEINS USING "*IN VITRO* VIRUS"

Naoto Nemoto and Hiroshi Yanagawa
Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194-8511, Japan

The strategy of assignment of a genotype to its phenotype is essential to molecular evolution. Recent advances in evolutionary molecular engineering have shown that the nature adopted three strategies of assignment of a genotype to its phenotype, i.e., "ribozyme-type", "virus-type" , and "cell-type". The ribozyme-type strategy is the most simple and effective because a single molecule carries both genotype and its phenotype on itself. However, it is difficult to select proteins by adopting the ribozyme-type. In the virus-type strategy, a genotype molecule (e.g. RNA) binds its phenotype molecule (e.g. protein) directly like non-enveloped viruses. On the other hand, a genotype molecule and its phenotype molecule are enclosed with a capsule in the cell-type strategy. In the affinity selsection of proteins, the virus-type strategy is more effective than the cell-type strategy. However a phage display method is insufficient to serach larger sequence spaces because a variety of displayed proteins is critically limited by the host cells infected with those viruses. Thus a virus-type strategy using an *in vitro* translation system is an alternative method. We made a "virus"-like molecule, so called "*in vitro* virus" that mRNA (genotype) bound to its encoded protein (phenotype) through a puromycin on a ribosome in a cell-free translation system (1). *In vitro* virus will enable us to select functional proteins in a manner of *in vitro* selections. We constructed two random libraries by random shuffling of segments and ligation of random sequences for searching larger protein sequence spaces. In the course of selecting functional proteins from these libraries using *in vitro* virus, we will find how proteins evolved in the early stage of molecular evolution and what a virus-like molecule like the "*in vitro* virus" played in the transition state from RNA world to RNP (RNA and protein) world (2).

(1) Nemoto, N, Miyamoto-Sato, E., Husimi, Y., & Yanagawa, H. (1997) *FEBS Lett.* **414**, 405-408
(2) Nemoto, N & Husimi, Y. (1995) *J. Theor. Biol.* **176**, 67-77

P3.1

TOWARDS THE SELECTION OF A MINIMAL REPLICASE

Alexander Azzawi and Günter von Kiedrowski
Lehrstuhl für Organische Chemie I, Ruhr-Universität Bochum,
44780 Bochum, Germany

The present generation of artificial self replicating systems typically suffer from product inhibition leading to parabolic growth and subsequently to survival of everybody. In recent years, two strategies were developed in our laboratory to overcome the problem of product inhibition. The first strategy utilizes irreversible immobilization of template molecules to surfaces, preventing template-product duplex formation (SPREAD).[1] The second strategy is to stabilize the ternary complex of a minimal replicator by a phosphate leaving group that is able to wrap around the ternary complex (see figure1). As long as the leaving group catalyzes the phosphoryl transfer step we understand such a leaving group as a minimal replicase.

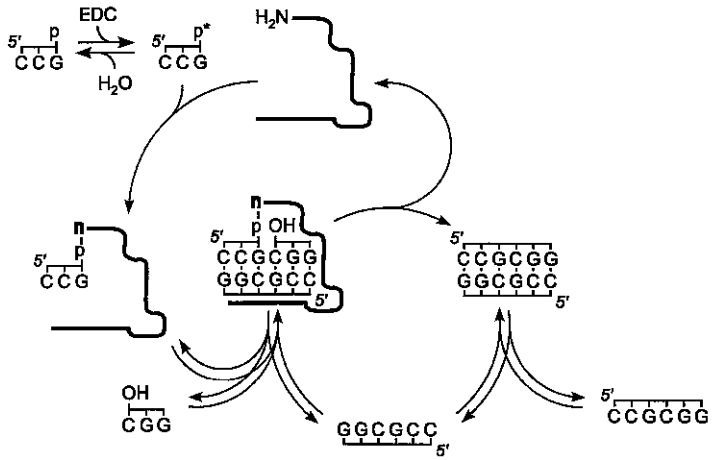


Fig. 1: Minimal Replicase

As a suitable leaving group we choose DNA since the self replicating system consists of oligonucleotides anyway. In vitro selection experiments were carried out in order to select a minimal replicase from a combinatorial library containing oligonucleotides with a 3'-5'-phosphoramidate linkage. So far our selection scheme resulted in the

P3.1 continued

Page 2 of: 'Torwards a minimal replicase' by Azzawi *et al.*

selection of oligonucleotides that were cleaved by hydrolysis in the presence of the cofactor pCGG.[2]

More recently we have been studying model reactions in which the template length was varied and the leaving group consisted of an 18mer amino-oligonucleotide having a defined sequence.

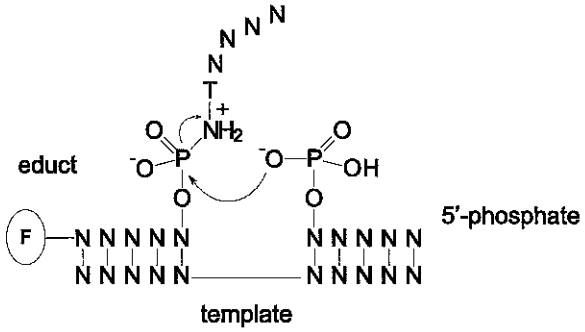


Fig. 2: Phosphoryl transferreaction

Our experiments show that phosphoryl transfer as outlined in figure 2 occurs instead of hydrolysis of the phosphoramidate bond if the template exceeds a critical length. Based on this results we have designed a new in vitro selection experiment using a N72 library as leaving group. This experiments are currently investigated.

[1] A. Luther, R. Brandsch, G. v. Kiedrowski, *Nature* **1998,** *396*, 245-248
[2] J. Burmeister, G. von Kiedrowski, A. D. Ellington, *Angew. Chem.* **1997**, *109*, 1379-1381, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1321-1324.

P3.2
INFLUENCE OF SEQUENCE EXTENSION ON SELF REPLICATING OLIGODEOXYNUCLEOTIDES

Jan Bülle and Günter von Kiedrowski
Lehrstuhl für Organische Chemie I, Ruhr-Universität Bochum,
44780 Bochum, Germany

Artificial self replicating systems based on dimers and trimers of modified oligonucleotides have been thoroughly investigated [1-3]. Because of product inhibition all these systems show parabolic growth and result in coexistance of competing species consequently. For a better understanding of the dynamic background of self replicating systems it is desirable to know more about the contiguity between thermodynamics and kinetics. The stepwise sequence extension of the DNA building blocks inevitably leads to higher complex stabilities. The longer the sequences are the more information can be transferred from one generation to the next in principle. Informational transfer is a major supposition for chemical evolution. Non-enzymatic template directed ligations of oligonucleotides with longer sequences have been described [4], dynamic replicators of that kind are unknown so far.

The stepwise extension of the self-complementary template sequence CCGCGG with A and T leads to three new self replicating systems of different thermodynamic properties.

The influence on the kinetic behaviour has been currently investigated.

[1] von Kiedrowski G., *Angew. Chem. Int. Edn Engl.* **1986**, *25*, 932-935
[2] Zielinski W.S., Orgel L.E., *Nature* **1987**, *327*, 346-347
[3] Sievers D., von Kiedrowski G., *Nature* **1994**, *369*, 221-224
[4] Joyce G.F., Orgel L.E., *J. Mol. Biol.* **1988**, *202*, 677-681

P3.3

ON THE CHEMISTRY OF THERMAL CONJUGATE PROTEINS

Peter R. Bahn
Bahn Biotechnology Co., RR2 Box 239A, Mt. Vernon, Illinois, USA 62864

Different covalently bound thermal metalloproteins, thermal phosphoproteins, thermal lipoproteins, thermal phospholipoproteins, thermal glycoproteins, and thermal nucleoproteins were made separately by heating 1:2:2:1 mixtures (by weight) of Conjugate : Glutamic Acid : Aspartic Acid : Basic - Neutral Amino Acids (equimolar mixture). The conjugate compounds employed were: $MgCl_2$, $CaCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$, KH_2PO_4, fatty acids, cholesterol, lecithin, glucose, and adenosine monophosphate.

All of the synthesized thermal conjugate proteins were biuret positive, ninhydrin positive, and soluble in SDS; could absorb Coomassie blue; and formed microspheres when heated in boiling water and allowed to cool.

Metal contents in the thermal metalloproteins were quantitatively determined by atomic emission spectroscopy to be respectively: 0.019% Mg, 0.028% Ca, 0.48% Fe, 0.0037% Co, 0.13% Ni, 0.87% Cu, and 0.017% Zn.

The synthesized thermal phosphoprotein, thermal phospholipoprotein, and thermal nucleoprotein tested positive for attached phosphate according to color analysis with $HClO_4$, $(NH_4)_6Mo_7O_{24}$, and Fiske-Subbarow reducer.

Synthesized thermal glycoprotein tested negative with Benedict's solution, indicating that it does not contain free monosaccharides; however, upon hydrolysis it gave a positive Benedict's test indicating that the polysaccharide moieties can be cleaved into monosaccharides.

Thermal conjugate proteins serve as models for the first conjugate biopolymers to evolve on the Primitive Earth.

Bahn,P.R. and Fox,S.W.:1996,CHEMTECH,Vol.26,p.26.

P3.6

FORMATION OF RNA OLIGOMERS BY MONTMORILLONITE CATALYSIS: A MODEL STUDY

Gözen Ertem* and James P. Ferris
Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180-3590 U.S.A.

Possible catalytic role of minerals in the formation of biomolecules in the primitive earth was first proposed by Bernal (1949). We have been studying the formation of phosphodiester bond in RNA oligomers using clay mineral montmorillonite as catalyst. In the presence of Na-montmorillonite, 5'-phosphorimidazolides of nucleosides, used as activated monomers, undergo self condensation in an aqueous electrolyte solution at pH 8 to produce oligomer chains with up to 12 monomer units in length. Regiospecificity, and the length of the longest oligomer formed in these reactions vary with the base moiety of the ribose (Ertem and Ferris, 1998; Kawamura and Ferris, 1996).

Oligocytidylates formed by montmorillonite catalysis serve as templates for the self condensation of activated guanosine monomers. Oligoguanylates formed on synthetic oligocytidylates linked by mainly, or exclusively, 2',5'-phosphodiester bonds contain 3',5'- as well as 2',5'-linkages (Ertem and Ferris, 1996).

We have also investigated the condensation of two different kinds of activated monomers in the presence of montmorillonite. Analysis of the reaction products revealed that pNpN type dimers form with a notable regio- and sequence selectivity. The same trend is observed in the dimers formed from the reaction of three and four kind of monomers (Ertem and Ferris, 1999). We have also demonstrated from the order of elution from the reverse phase column (Kanavarioti, 1998) that hetero-trimers along with homo trimers of the pNpNpN type form in these reactions.

These model studies demonstrate that catalysis may have played a significant role for the formation of phosphodiester bonds in aqueous solution in the primitive earth.

Bernal, J. D.: 1949, Proc. Physical Soc., Section A, **62**, 537-558.
Ertem, G. and Ferris, J. P.: 1996, Nature **379**, 238-240.
Ertem, G. and Ferris, J. P.: 1998, Viva Origino **26**, 203-218, review paper.
Ertem, G. and Ferris, J. P.: 1999, submitted for publication.
Kanavarioti, A.: 1998, J. Molec. Evol. **46**, 622-632.
Kawamura, K. and Ferris, J. P.: 1996, Origins Life Evol. Biosphere submitted.

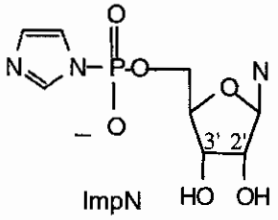
* Present address: Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20007 U.S.A.

P3.7

BRIDGING THE PREBIOTIC AND RNA WORLDS; PREBIOTIC SYNTHESES ON MINERALS

Gözen Ertem, K. Joseph Prabahar, Prakash C. Joshi and James P. Ferris, Dept. of Chemistry, Rensselaer Polytechnic Inst., Troy NY USA, 12180

The condensation of RNA monomers to RNA is one of the basic tenets of the RNA world scenario for the origin of life (Gilbert 1986). We discovered that montmorillonite clay catalyzes the condensation of activated mononucleotides to oligomers in pH 8 aqueous solution at room temperature (Ferris and Ertem 1993) (1).



ImpN

N= A, G, C, U, I

MONTMORILLONITE, Mg^{2+}

pH =8, 25°C, ~4 days

$(pN)_2$-$(pN)_{15}$ (1)

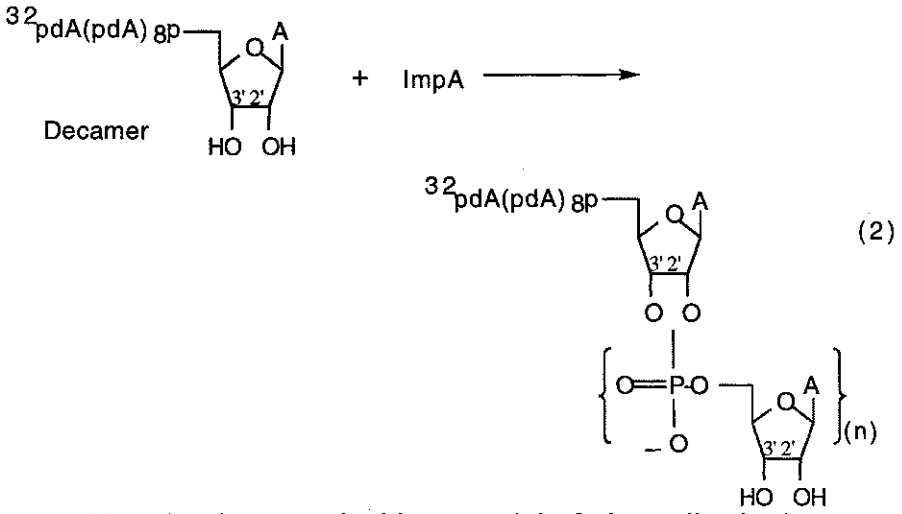
2',5' and 3',5,-phospho-diester bonds and pyrophosphate links. Linear and cyclic oligomers formed.

It is postulated that in the RNA world scenario that sequence information in the RNA was preserved by template-directed synthesis. This proposal was tested by investigation of the synthesis of oligo(G)s on the heterogeneous oligomers formed by clay mineral catalysis. Oligo(C) templates which contain mainly 2',5'-links together with small amounts of 3',5'-links, cyclic oligomers and pyrophosphate links, directed the synthesis of the complementary oligo(G)s (Ertem and Ferris 1997). This experiment demonstrated that the information content of the RNA formed by prebiotic processes could have been maintained by template-directed synthesis on the primitive Earth.

Another key aspect of life in the RNA world is the catalysis of chemical processes by RNA (ribozymes). It is unlikely that RNA 6-15 monomer units in length, formed by montmorillonite-catalyzed condensation of activated monomers, would exhibit catalytic activity. It is possible to make oligo(A)s as long as 50 mers by adding activated monomer daily to a decameric primer for 14 days (Ferris et al. 1996) (2). It is proposed that an RNA containing 30-60 units would have been long enough to have catalyzed the reactions of other RNA molecules (Szostak and Ellington 1993) and would have replicated with adequate fidelity to maintain its information content (Joyce and Orgel 1993). Consequently, this type of primer elongation reaction may have been a prebiotic route to longer RNAs.

P3.7 continued

(Prebiotic synthesis on minerals continued)



(2)

New developments in this research include studies in the concurrent reaction of two or more activated nucleotides on montmorillonite. Significant sequence- and regio-selectivity was observed in these reactions demonstrating that a random mixture of all possible structures are not formed in the montmorillonite-catalyzed reaction. The primer-initiated montmorillonite-catalyzed synthesis of longer RNAs, with two or more different monomer units, will also be described. The reactivity in the 1-methyladenine activated mononucleotides (Prabahar and Ferris 1997) in template-directed synthesis and primer elongation will be discussed and new findings on the reaction pathway for the montmorillonite-catalyzed formation of RNA will be presented.

Acknowledgments: This work was supported by NSF grant CHE-9619149 and NASA NSCORT grant NAG5-7598.

Ertem, G. and Ferris J. P. :1997, J. Am. Chem. Soc. **119**, 7197-7201.
Ferris, J. P. and Ertem, G. :1993, J. Am. Chem. Soc. **115**, 12270-12275.
Ferris, J.P, Hill, A.R., Jr., Li, R., Orgel, L.E.:1996, Nature **381**, 59-61.
Gilbert, W. :1986, Nature **319**, 618.
Joyce, G. F., Orgel, L.E. :1993, In: Gesteland, R. F, Atkins, J. F. (eds) The RNA World. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 1-25
Prabahar, K. J., Ferris, J. P. :1997, J. Am. Chem. Soc. **119**: 4330-4337.
Szostak, J. W., Ellington, A. D. :1993, In: Gesteland, R. F., Atkins, J. F. (eds) The RNA World. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 511-533

P3.8

KINETICS OF dG5'ppdG SYNTHESIS AS A FUNCTION OF MONOMER AND POLY(C) CONCENTRATION: INSIGHTS INTO THE TEMPLATE-DIRECTED MECHANISM.

Gangopadhyay, Sumana[1] and Kanavarioti, Anastassia
Department of Chemistry and Biochemistry, UCSC, Santa Cruz, CA 95064, USA

The formation of the pyrophosphate-linked dideoxyguanylate, dG5'ppdG, from deoxyguanosine 5'monophosphate 2-methylimidazolide, 2-MeImpdG, was studied in aqueous solution at 23° C, pH 7.8, 1.0 M NaCl and 0.2 M Mg^{2+} in the presence and absence of polycytidylate, poly(C). Analysis by high-performance liquid chromatography (HPLC) indicates that 2-MeImpdG hydrolyzes to deoxyguanosine 5'monophosphate, 5'dGMP, and self-condenses to dG5'ppdG (Kanavarioti, 1998). In the absence of poly(C) the initial rate of dG5'ppdG formation, d[D]/dt in Mh^{-1} determined by timed HPLC analysis, shows a 2nd order dependence on monomer concentration and a leveling off with [2-MeImpdG] > 0.07 M. These results indicate a bimolecular dimerization that becomes 1st order, possibly due to formation of stacks of molecules with the higher guanosine concentration.

In the presence of poly(C) the formation of the dimer is accelerated and oligoguanylate products are formed by dG5'ppdG elongation with 2-MeImpdG molecules. Here, values of d[D]/dt were determined as a function of monomer as well as poly(C) concentration. With 0.10 M 2-MeImpdG and in the range 0.004 M ≤ [poly(C)] ≤ 0.025 M a linear relationship between d[D]/dt and poly(C) concentration suggests a template-directed mechanism of dimerization. At lower 2-MeImpdG concentration (<0.1 M) the kinetics become more complex but can still be accounted for by the proposed mechanism. In conclusion, the poly(C)/2-MeImpdG system provides a textbook example for the kinetic behavior of a template-directed dimerization.

[1]On leave of absence from the Department of Chemistry, Gurudas College, Calcutta, India

Kanavarioti, A.: 1998, J. Org. Chem. **63**, 6830-6838.

P3.9

EVOLUTION OF NUCLEIC ACID COMPLEXITY: DINUCLEOTIDES IN THE GENETIC CODE AND 5S rRNA

Romeu C Guimarães[1], Regina HR Ferreira[2]
([1]Dept. Biologia Geral, Inst. Ciências Biológicas (ICB), Univ. Federal Minas Gerais (UFMG), 31270.901 Belo Horizonte MG Brazil; Fax +55-31-499.2567, romeucg@icb.ufmg.br, www.icb.ufmg.br/~romeucg; [2]Univ. Tiradentes, CCBS, 49030.270 Aracaju SE Brazil)

Genetic code. Anticodonic core dinucleotide (diN) types (homogeneous, Ho, RR and YY; mixed, Mx, RY and YR) were correlated (91-70% consistency) with aminoacyl-tRNA synthetase (aaRS) classes 2 and 1, respectively (RC Guimarães 1998 Genetic code: hydropathic, dinucleotide type and aminoacyl-tRNA synthetase class organization. In: *Exobiology - matter, energy, and information in the origin and evolution of life in the universe*, Ed. J Chela-Flores and F Raulin, Kluwer, Dordrecht, Holland, p 157-60). We now report that diN complementarity was less constrained in Ho, aa attributed to Mx diN lacked the acidics, and that measures of complexity were higher in the Mx1 than in Ho2 correspondences: Mx diN and amino acid (aa) hydropathic regression (11 assignments) was tuned in a linear gradient while Ho diN regression depicted extreme hydropathy clusters (8 assignments) plus 4 outliers (Gly, Pro, Ser); aa sizes were higher in the YR than in the YY quadrant; degeneracy was higher in the Ho2 axis and all punctuation signs belonged in the Mx1 axis. In the 5S rRNA of fungi, Mx diN were more abundant than expected and Ho diN avoided (RHR Ferreira 1997 PhD Thesis, ICB-UFMG; 75% consistency). Mx diN frequencies increased from primitive to complex fungi, and Ho diN decreased, as had been shown (RC Guimarães and VA Erdmann 1992 Evolution of adenine clustering in 5S rRNA. Endocyt. Cell Res. 9: 13-45) for adenine clusters in all organisms complexity and simplification routes.

Observation of the same phenomenon in such distinct functional domains indicated that this is a general property of nucleic acids, being rich in monotonous runs of bases when involved with simpler structures and functions, allowing more plasticity and ambiguity, and in oligonucleotides of heterogeneous (RY, YR) composition when developing more complex functions, with more specific interactions. (Acknowledgments: CNPq, FAPEMIG)

P3.10

AVERAGE DINUCLEOTIDE AND AMINO ACID RESIDUE HYDROPATHY SCALES FIT THE GENETIC CODE CORRELATION

Romeu C Guimarães[1], *Carlos HC Moreira*[2]
([1]Dept. Biologia Geral, Inst. Ciências Biológicas, Univ. Federal Minas Gerais, 31270.901 Belo Horizonte MG Brazil, Fax +55-31-499.2567, romeucg@icb.ufmg.br; [2]Dept. Matemática, Inst. Ciências Exatas)

Hydropathic correlation in the genetic code (RC Guimarães 1998 Genetic code: hydropathic, dinucleotide type and aminoacyl-tRNA synthetase class organization. In: *Exobiology - matter, energy, and information in the origin and evolution of life in the universe*, Ed. J Chela-Flores and F Raulin, Kluwer, Dordrecht, 157-60) was based on average Studentized residuals from linear regressions. We now offer for further tests and applications the correspondent average normalized hydrophilicity scales of dinucleotides (diN) and amino acid (aa) residues. Original diN (2 scales) and aa molecule (7 scales) were obtained from Lacey and Mullins (1983, Orig. Life Evol. Biosph. 13: 3-42) plus Roseman (1988; 2 scales) and 8 aa residue scales from Turner and Weiner (1993; 1995, pers. comm.). DiN: AA 0.000 AG 0.059 AC 0.189 AU 0.199 GA 0.098 GG 0.196 GC 0.403 GU 0.433 CA 0.312 CG 0.564 CC 0.928 CU 1.000 UA 0.303 UG 0.596 UC, UU 0.931. Aa residues (GMR): Ala 0.258 Arg 0.982 Asn 0.809 Asp 0.962 Cys 0.111 Gln 0.841 Glu 0.935 Gly 0.423 His 0.573 Ile 0.029 Leu 0.066 Lys 1.000 Met 0.059 Phe 0.000 Pro 0.677 Ser 0.508 Thr 0.438 Trp 0.325 Tyr 0.361 Val 0.069. Correlation coefficients were 0.905 with the 11 mixed diN (RY, YR) assignments, and 0.931 with these plus the 8 homogeneous diN (RR, YY) assignments (excluding Gly, Pro and Ser, which were confirmed as outliers). Our previous indication that scales for aa residues were better than those for aa molecules was confirmed. The average of 15 scales of aa residues and molecules (excluding those of aa molecules from Garel et al (1973) and Weber and Lacey (1978), which departed widely from the others) yielded higher correlation coefficients but failed in indicating the outlier position of Pro and indicated this for Arg CG (Cook's residuals test). Individual best ranking scales, evaluated by comparison with GMR were: AMPO7 (Degli Esposti et al, 1990) > Rao and Argos (1986) > Rose and Dworkin (1989) > Kyte and Doolittle (1982), but none of these depicted all genetic code regularities as did GMR. (Acknowledgments: CNPq, FAPEMIG)

P3.11

HYDROLYSIS OF 3',5'- AND 2',5'-LINKED DINUCLEOSIDE MONOPHOSPHATE IN AQUEOUS SOLUTION AT 175 - 240 °C

KAEDE Chika and KAWAMURA Kunio
Department of Applied Chemistry, College of Engineering,
Osaka Prefecture University, Sakai, Osaka, JAPAN 599-8531

It is widely believed that RNA is the molecule that had first genetic information on the earth. On the other hand, hydrothermal environment is though to be contribute for the formation of biomonomers and biopolymers. Thus, it is necessary to investigate the formation and decomposition of RNA in aqueous solution at high temperatures. Recently, a new monitoring method for rapid reactions in aqueous solution at high temperatures has been developed (Kawamura, 1998, 1999). In this study, we have studied kinetics of decomposition of 3',5'- and 2',5'-adenylyladenosine monophosphate ($A^{3'}pA$ and $A^{2'}pA$) at 175 - 240 °C using the new monitoring method.

The decomposition reactions of $A^{3'}pA$ and $A^{2'}pA$ were monitored in aqueous solution containing 0.1 M NaCl, 0.05 M $MgCl_2$, 0.05 M imidazole (pH=8.0) at 175 - 240 °C. Formation of 3'-adenosine monophosphate (3'-AMP), 2'-AMP, 2',3'-cyclic-AMP, adenosine, and A2'pA were detected in the reaction of A3'pA by reversed-phase HPLC. Thus, the hydrolysis of A3'pA is expressed by equation (1) - (2).

$A^{3'}pA$ -> 2',3'-cyclic-AMP + adenosine (1)
2',3'-cyclic-AMP -> 3'-AMP + 2'-AMP (2)

Further the interconversion between $A^{3'}pA$ and $A^{2'}pA$ were observed. The rate constants of disappearance of $A^{3'}pA$ and $A^{2'}pA$ were determined in imidazole or HEPES buffer solutions, in which the rate constants in imidazole buffer were somewhat smaller than those in HEPES buffer at pH 8. The activation energy of the hydrolysis of $A^{3'}pA$ is 74 kJ mol^{-1} in imidazole and 75 kJ mol^{-1} in HEPES buffer.

The hydrolysis rate of $A^{2'}pA$ was about 2 - 9 times faster than that of $A^{3'}pA$ at 175 - 240 °C in pH 6 - 8 solutions and the activation energy $A^{2'}pA$ was 45 kJ mol^{-1}. This fact suggests that 2',5'-linked RNA is more stable than 3',5'-linked RNA in neutral pH at high temperatures. The stability of dinucleoside monophosphate with other types of nucleotide bases are being investigated at present.

Kawamura, K. : 1998, Nippon Kagaku Kaishi, **1998**, 255.
Kawamura, K. : 1999, Chem. Lett., **1999**, in press.

P3.12

CHEMICAL EVOLUTION OF RNA IN AQUEOUS SOLUTION AT HIGH TEMPERATURES : A NEW METHOD FOR MONITORING OF HYDROTHERMAL REACTIONS

KAWAMURA Kunio
Department of Applied Chemistry, College of Engineering,
Osaka Prefecture University, Sakai, Osaka, JAPAN 599-8531

Discovery of the catalytic activity of ribonucleic acids (RNA) have suggested that RNA played an important role in the first life on the earth. On the other hand, hydrothermal environment in the primitive ocean has been thought to significantly contribute to the emergence of life. Thus, it is important to study the chemical evolution of RNA under hydrothermal environments. Although RNA is generally believed to be labile at high temperatures, quantitative analysis has not been carried out. One reason is that monitoring of fast reactions in aqueous solution at high temperatures was normally difficult or impossible by batch reactors.

To solve the problem, we developed a new monitoring method for hydrothermal reactions using flow tube reactor, in which the monitoring of hydrolysis of adenosine 5'-triphosphate (ATP) was succeeded at 125 - 300 °C in 0.37 - 140 s (Kawamura, 1998). The scope of this method has been investigated and a remarkable improvement was recently achieved using fused-silica capillary tubing, which allows monitoring hydrothermal reactions in the time range 3 - 1000 ms (Kawamura, 1999). This is the most rapid monitoring method for hydrothermal reactions. The method enables to monitor the formation and decomposition of RNA in aqueous solution at high temperatures in a millisecond time scale.

Using this method, kinetics of the consecutive hydrolysis of ATP to hypoxanthine and the decomposition of dinucleotide monophosphate have been studied in aqueous solution at 125 - 300 °C. The rate constants and activation energy were determined and the reaction rate and pathway were compared with enzymatic degradation of RNA. Basing on these studies, the possibility of the RNA world at high temperatures is discussed. The application of this monitoring method for the formation of RNA is being investigated at present. This method enables monitoring of more general reactions on the chemical evolution such as peptide formation, in principle.

Kawamura, K. : 1998, Nippon Kagaku Kaishi, **1998**, 255.
Kawamura, K. : 1999, Chem. Lett., **1999**, in press.

P3.13

EVOLUTION OF GENES

O.P.H. Khandelwal, 101, Happy Apartment, 16 E, Sadhna Nagar,
Indore - 452005 (M.P.), INDIA, e-mail : o_khandelwal@hotmail.com

Key Words: Pre RNAworld, RNA-World, Origin of life, DNA-world, Self ligation, Protein-World, Thioseter-World, Ribonucleopeptide world.

The question of genes evolution is still unsolved. Amino acids were the first precursors (Miller, 1953) of life. These amino acids and other chemicals HCN form purines and pyrimidines (Ferries, 1967,1995, 1997). Orgel (Orgel, 1968, 1989, 1991) obtained first monomer of nucleic acids. Gilbirt (1986) proposed "RNA world" and "pre RNA world" by Joyce and others (Joyce, 1989). Now we have doubt about RNA world, DNA world is also proposed (Miller, 1996). Now we can't think life without DNA. Recently Joyce (1994, 1995, 1997) and his colleges have shown the catalytic activity of DNA. It is surprising achivement because 10 years ago we were not able to even consider DNA as a catalytic agent (khandelwal, 1989). How DNA is related to evolution of first living being? Theoretically we will see that it may happen under the plausible conditions and different pH, temprature and salt concontration, with the help of self ligation and catalyzing activity. How gene came into existance?

Life may have transits from pre RNA world to RNA world to "Ribonucleopeptide world" (Di Giulio, 1997) and finally DNA wold to Protein world. Thioester world (de Duve, 1995) may also joined before DNA world. de Duve has suggested that thioester world in early stage of development of life could have provided energetic and catalytic frame work of the protomatabolic set of premitive chemical reaction that led to form the first building block of life to RNA world and subsequently sustained RNA until metabolism took over.

The ribonucleopeptide world and thioester world might have linked to envolve the primitive biomolecular genetic system (BGS, Altestein, 1996) and replicate and translated on the basis of progene. The progene might have acted as catalytic agent and provided the basis for evolution of gene.

P3.13 continued

Altestein; A.D. (1996), Poster 118, ISSOL 96, Book for Programme & Abstracts P-112.

Di Giulio M., (1997), J. Mol Evol. 45 : 571 - 578.

de Duve. C. (1995) American Scientist, Vol. 83 : Sept-Oct. 1995 : 427 - 437.

Ferris, J, et.al. (1967), J. Mol. Boil. 30. 223 - 253.

Ferris J.P., (1995) A comprehensive Desk Reference (Ed.) Robert A Meyers. P. 288 - 297, and 1995, VCH, Publisher, P. 628 - 631.

Ferris J.P., et.al (1997) J.Am. Chem. Soc. 119; 7197 - 7201.

Gibson T.J., et.al.(1990) J. Mol. Evol. 1990, 30; 7 - 15.

Gilbert, W. (1986) Nature 319; 618

Gilbert, W. (1987) Cold spring harbor symposium on quantative Biology. 52; 901 - 905.

Joyce G.F., et.al. (1987), Pro. Nat. Acad. Sci USA, 84; 4398 - 4402.

Joyce G.F. (1989) Nature Vol. 338 No. 6212; 217 - 224.

Joyce G.F., (1991) The New Biologist Vol. 3 No.4; 399 - 407.

Joyce G.F. et.al. (1994) Chemistry and Biology December 1994 1; 223 - 239.

Joyce G.F. (1995) Chemistry and Biology 2; 655 - 660.

Joyce G.F. et.al. (1997) Proc. Nat. Acad. Sci. USA, Vol.94; 4262 - 4268.

Khandelwal O.P. (1989) Origins of Life and Evol. of Biosphere Vol.19 (3-5); 354 - 355.(Abstract)

Personal Comments (1989) obtained by author from Dr. G.F. Joyce on DNA self liagation and catalytic agent.

Miller S.L. (1953) Science, 117; 528 - 529.

Miller S.L. (1997) Nature Structure Biology Vol.4 No.3 March 1997; 167 - 169.

Miller S.L. (1996) Poster 74 ISSOL 96, Book for Programme & Abstracts P-93.

Orgel. L.E. (1989) J.Mol.Evol. 29, 465 - 473.

Orgel L.E. (1991) J.M.Evol. 32; 274 - 277.

Orgel L.E. (1968) J. Mol. Biol. 33; 693-704.

P3.14

SELF-SUPPORTING REPLICATION AND PROTOMETABOLISM AS A RESULT OF BIFURCATION

Vladimir N. Kompanichenko ,Complex Analysis of Regional Problems Institute, Birobidjan 682200, Russia; Present address: DVIMS, CARPI, 31 Gerasimov str., Khabarovsk 680021, Russia

All models of pre-biological microsystems in accordance with the main directions in protobiochemistry can be grouped into two classes. In one of them, the compartmentalization, the microsystems of proteinoide composition divided by the inner membrane compartment (coacervates, proteinoide microspheres, marigranules, etc.), are considered as primary models for protocells. Models of «RNA-World» represent primary chains of nucleotides or their analogues. Microsystems of both classes possess a certain features of internal and external activity. For instance, coacervates are able to grow and selectively assimilate substance, in proteinoide microspheres the catalitic activity become apparent, macromolecules of RNA-World under certain conditions are able to self-replication. However, we have to make the following conclusion: all chemical microsystems without exception have one common property - soon after their formation the features of internal and external activity are fading away in them or for some time they can be maintained with help of experimenter. In fact, the transition from chemical to protobiological evolution evidently assumes no fading of primary features of microsystems activity but on the contrary their strengthening with the following transformation into self-maintaining dynamic processes (protometabolism, natural self-replication).

A major thesis of the author's conception is that the chemical evolution had to be interrupted by the act of spontaneous self-organization under the *strongly* non-equilibrium conditions, i.e. by bifurcation, for life to appear. It is based on the fundamental notions of non-equilibrium thermodynamics. Bifurcation occurs when the amplitude of fluctuations in the environment exceeds a certain critical level. At this moment, between all molecules of a system, there arises a long-range correlation, i.e. an integrated organization of a system. In the ocean and even near the hydrothermal vents on its bottom the scale of fluctuations is not powerful. The conditions here are oscillating from equilibrium to weakly non-equilibrium. Strong fluctuations of physico-chemical and thermo-dynamical parametres (T, P, C, pH, Eh) manifest themselves only in hydrothermal systems

P3.14 continued

SELF-SUPPORTING REPLICATION AND PROTOMETABOLISM AS A RES...

below the Earth's surface (actually up to 3-4 km). It is this area that the author considers as an incubation zone where the primary forms of protolife were originated. A decisive step in life origin was made during the bifurcational transformation of pre-cellular organic microsystems (microspheres) that consisted of polymers of both classes. As a result, in microspheres there appeared autofluctuating and then self-maintaining cyclic processes, integrated organization and active exchange with the environment. The author proposes to call this kind of transformed microsystems as quasibionts. Appearance of cyclic reactions in quasibionts led to strengthening of interaction between aminoacids and nucleotides, that had further resulted in the formation of self-replicating probionts. In the process of transformation of quasibionts into probionts a complementary type of relation was formed in polynucleotides chains. Development of the protogenetic structures was caused by the necessity of further improvement of the «administrative apparatus» in quasibionts, evoluted «from chaos to order». It is this moment of arising interaction between polypeptide and polynucleotide chains with their alternative types of relations that is considered as initial one for the origin of protolife. Further, processes of protometabolism and self-replicationin probionts were activated by the environmental fluctuations. Originated in the depth of hydrothermal systems quasibionts and probionts were then removed by rising streams of solutions to hydrothermal vents on the ocean bottom where protoecosystems started to form. Near the hydrothermal vents the long-term evolution of probionts resulted in progenotes already possessing the features of primary species and then to the formation of stable species of Archaea and Bacteria hyperthermophiles some of them preserved till our days.

Kompanichenko, V.N. :1996, *Hydrothermal Origin of Life in Earth Depth,* PGS, Khabarovsk, p. 94
Kompanichenko, V.N. :1996, Nanobiology **04**

P3.15

IN VITRO EVOLUTION OF DOUBLE-STRANDED DNA-CLEAVING RIBOZYMES

Roshan M. Kumar and Gerald F. Joyce
The Scripps Research Institute, La Jolla, CA 92037

Catalytic RNAs are thought to have played a crucial role in the development of terrestrial life. As such, defining the limits of the catalytic potential of RNA is relevant to the RNA world hypothesis. Furthermore, novel ribozymes might have practical applications in the modern world. We are applying the tools of directed molecular evolution to the problem of generating catalytic RNA molecules that are capable of strand invasion into duplex DNA, followed by the sequence-specific cleavage of one of the two DNA strands. Such molecules would be "programmable" restriction enzymes that might be useful for a variety of research and therapeutic purposes.

We generated a library of RNAs based on evolved variants of the group I ribozyme as a starting point for the evolution of the desired catalytic behavior. The group I ribozyme cleaves single-stranded RNA in its natural context, and has been selected over 63 generations of *in vitro* evolution to recognize and cleave single-stranded DNA (Tsang & Joyce, 1996). We aim to provide this DNA-cleaving ribozyme with strand invasion activity that would allow it to recognize and cleave a target duplex DNA substrate. Because this is likely to be a difficult task, we are first aiming to select for molecules that can cleave an intermediate substrate, neither fully single-stranded nor double-stranded, but containing a "bubble" of abasic nucleotide analogues within one strand of the duplex.

We are applying selection pressure to the evolving population of ribozymes by progressively decreasing the size of the bubble and controlling the reaction conditions to favor the emergence of a strand-invading phenotype. Coupled with the continued introduction of diversity into the population through *in vitro* mutagenesis, we aim to evolve ribozymes that are capable of cleaving a native DNA duplex.

Tsang, J. and Joyce, G.F. : 1996, *J. Mol. Biol.* **262**, 31.

P3.16

IN VITRO EVOLUTION OF A DNA ENZYME WITH DEGLYCOSYLASE ACTIVITY

P. Ordoukhanian, T. Sheppard, and G. F. Joyce
Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, U.S.A.

Deoxyribonucleic acids have been shown to exhibit catalytic activity similar to their RNA counterparts. We evolved a DNA enzyme that is capable of producing an abasic site in DNA through depurination of a target guanine. The cleavage mechanism appears to involve a pK shift of the N7 of guanine, with an optimum rate for the reaction at pH 4.9. The DNA enzyme has a k_{cat} of 0.2 min^{-1} in the presence of 2 mM Ca^{2+} at pH 5.2. In comparison, the uncatalyzed rate of reaction under the same conditions was 4×10^{-7} min^{-1}. Thus, the DNA enzyme exhibits a 550,000 fold rate enhancement. The DNA enzyme was redesigned to operate in an intermolecular format, giving a k_{cat} of 0.018 min^{-1} and a K_m = 5.4 μM at 25° C ($k_{cat}/K_m = 3.3 \times 10^3$ M^{-1} min^{-1}). The identity of the cleaved products and the released guanine were confirmed by HPLC and mass spectrometry. The deglycosylase DNA enzyme demonstrates an activity that would have been important in maintaining the integrity of early genomes.

P3.17

INCREASING THE CHEMICAL REPERTOIRE OF NUCLEIC ACID ENZYMES THROUGH OLIGONUCLEOTIDE-TETHERED COFACTORS.

John S. Reader and Gerald F. Joyce
The Scripps Research Institute, La Jolla, CA 92037

Although nucleic acids are capable of catalyzing a wide range of chemical reactions, these enzymes lack the diversity of functional groups available to proteins. The absence of these groups may account for the low substrate affinity and/or poor reaction rates exhibited by many nucleic acid enzymes. In the "RNA world", one way of overcoming a restricted chemical repertoire would be the involvement of aminoacyl or peptidyl cofactors which, when bound to a nucleic acid, could participate in catalysis. These cofactors tethered to oligonucleotide "handles" would be easily recognized through Watson-Crick base pairing, simplifying the difficult task binding a cofactor with high affinity and also orientating the bound cofactor for catalysis. Aminoacyl or peptidyl oligonucleotides might have then led to the development of adaptor molecules that became involved in protein synthesis.

To test this hypothesis, regarding oligonucleotide cofactors, an *in vitro* evolution experiment has been initiated using a pool of DNA molecules in the presence or absence of aminoacyl oligonucleotides and selecting for molecules capable of hydrolysis of a peptide bond. The functional groups of histidine and glutamic acid were chosen for the aminoacyl oligonucleotides because these residues often play prominent roles in the catalytic mechanism of various protease enzymes. A nucleic acid enzyme has previously been shown to catalyze the cleavage of an amide bond, but with a rate enhancement of only 100-fold over background levels (Dai *et al.*, 1995). Consequently, it should be possible to probe the effect of introducing novel functionalities to nucleic acids for the catalysis of an energetically difficult reaction.

Dai, X., De Mesmaeker, A. & Joyce, G. F. (1995) Science **267** 237-239

P3.18

A HETEROGENEOUS SELF-REPLICATING RNA/PEPTIDE CYCLE

Matthew Levy and Andrew D. Ellington
Institute for Cellular and Molecular Biology,
University of Texas at Austin, Austin, TX 78712

Studies of self-replicating systems are of great interest to the field of the origin of life in that they shed light on one of the earliest periods in evolution. Previous studies have focussed on the template polymerization and cross-templating of activated RNA, DNA and PNA monomers (Orgel, L.E. 1992; Böhler C. *et al.* 1995; Schmidt J.G. *et al.* 1997;) as well as the auto-catalytic and cross-catalytic parabolic growth curves of short oligonucleotides (Sievers D. and von Kiedrowski, G. 1994). These experiments lend support to the idea that the RNA world may have been preceded by a simpler pre-RNA world, dominated by small self-replicating molecules with the evolution of more complex ribozymes arising later.

In addition to the nucleic acids and their anologues, short peptide sequences have also been found that can self-replicate forming both auto-catalytic and cross-catalytic cycles via template directed ligation (Lee D.H.*et al.* 1997). While it seems clear that the chemical nature of the 20 amino acids would exclude these replicators as the forebearers of the first genetic material, the fact that relatively small peptides can posses "catalytic" activity remains very intriguing.

In an effort to further understand the workings of self-replicating systems we have begun to investigate a hybrid self-replicating system involving both RNA and peptide replication.

In this system we have begun to test the ability of an RNA aptamer to serve as a catalyst (template) for the synthesis of its ligand, a peptide, from two smaller pieces. In addition we have begun to investigate the ligand's ability to serve as a template for the aptamer, thus setting up a heterogeneous cycle in which RNA makes peptide makes RNA. Such a system although much simpler, is not completely dissimilar to that currently found in biology in which DNA makes RNA makes protein makes DNA. Our results will be presented and disscussed.

Böhler C. Nielsen P.E. and Orgel L.E.: 1995, Nature **376**, 932.

P3.18 continued

A HETEROGENEOUS SELF-REPLICATING RNA/PEPTIDE CYCLE

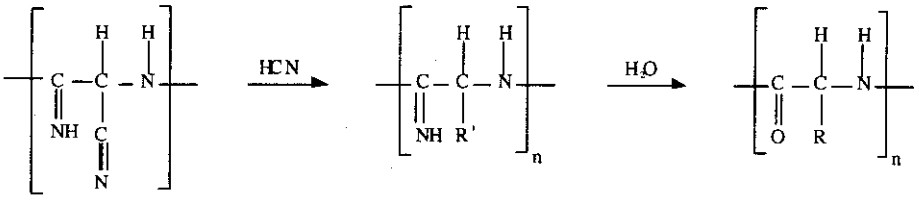
Lee D.H., Severin K., Yokobayashi Y. and Ghagiri M.R.: 1997, Nature **390**, 591.
Orgel, L.E.: 1992, Nature **358**, 203.
Schmidt J.G., Christensen L., Nielsen P.E. and Orgel L.E.: 1997, Nucleic Acids Res. **25**, 4792.
Sievers D. and von Kiedrowski, G.: 1994, Nature **369**, 221.

P3.19

THE HCN WORLD: ESTABLISHING PROTEIN–NUCLEIC ACID LIFE

Clifford N. Matthews
Department of Chemistry, University of Illinois at Chicago
Chicago, Illinois 60680, USA

Hydrogen cyanide polymers--heterogeneous solids varying in color from yellow to orange to brown to black--could be major components of the dark matter observed on many bodies in the outer solar system, including asteroids, comets, moons, rings and planets. Returned sample missions now in progress (such as project Stardust) may well show the presence of these polymers and their products in cometary and other extraterrestrial material. Current studies of these ubiquitous compounds point to the presence of polyaminomalononitrile (I), a polyamidine structure built only from hydrogen cyanide. Cumulative reactions of HCN (or other reactive species) with the activated nitrile groups of I yield other polyamidines (II, with side chains R') which are converted stepwise by water to polypeptides (III, with side chains R):



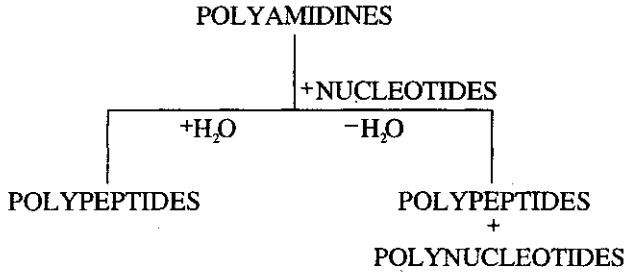
I II III

Overall, this series of reactions constitutes a route for the direct synthesis of polypeptides from hydrogen cyanide and water without the intervening formation of α-amino acids. Additionally, pyrolysis of cyanide polymers yields nitrogen heterocycles with purine and pyrimidine structures.

P3.19 continued

THE HCN WORLD: ESTABLISHING PROTEIN–NUCLEIC ACID LIFE (Page 2)

Implications for prebiotic chemistry are profound. Primitive Earth may have been covered by HCN polymers as well as other organic compounds through bolide bombardment or by photochemical reactions in a reducing atmosphere. According to this model, membrane material--carboxylic acids, carbohydrates, polypeptides--accumulated in lakes and oceans, while on land polyamidines could have been the original dehydrating agents directing the synthesis of nucleosides and nucleotides from available sugars, phosphates and nitrogen bases. Most significant would have been the parallel synthesis of polypeptides and polynucleotides arising from the dehydrating action of polyamidines on nucleotides:



Metabolic material--hardware--thus arose separately from genetic components--software--as proposed by Freeman Dyson. Subsequent interfacing, perhaps with the help of clays, then produced the first replicating protocells.

On our dynamic planet this polypeptide-polynucleotide symbiosis mediated by polyamidines may have set the pattern for the evolution of protein-nucleic acid systems controlled by enzymes, the mode characteristic of life on Earth today.

P3.20

ULTRASTRUCTURAL EVIDENCES OF TRANSITIONAL CELLS BETWEEN PROKARYOTES AND EUKARYOTES

Ernesto Palacios-Prü and Vicente Marcano. Electron Microscopy Center, University of the Andes, P. O. Box 163, Mérida, Venezuela. E-mail: prupal@ing.ula.ve

Further ultrastructural studies of different fungi species (Cedeño & Palacios-Prü 1990, 1992, 1996) revealed a discontinuity of the nuclear envelope which was not correctly interpreted. In present-day these structures are recognized by us as long apertures or passages lacking a diaphragmatic mechanism. These apertures (up to 1500 nm in diameter) may facilitate the free movement of the genetic material and molecules from the interior of the nucleus to the cytoplasmic region. In vertebrates, transport of molecules is carried out through nuclear pores. These pores are constituted by a complex of protein structures that regulate bidirectionally the traffic of molecules between the nucleus and cytoplasm either by free difusion or active transport (Pante & Aebi 1994, Gorlich 1997, Nigg 1997, Ohno et al. 1998). This finding of the long apertures in the nuclear membranes of lower eukaryotes led to reconsider the position of these organisms either as prokaryotes or "facultative" eukaryotes. Several nuclear phenotypes have been observed among lower eukaryote cells, including green algae and lichenized and non-lichenized fungi species. These nuclear phenotypes are distinguished mainly by the presence or absence of nuclear apertures, nuclear pore complex and DNA separated or not from ribosomes.

The species examined in this study were: *Cladonia calycantha* Del. ex Nyl. (lichenized Ascomycotina); *Fusarium* sp. (non-lichenized fungus); *Trebouxia* sp. (green algae) and *Rhizoctonia solani* Kuehn (non-lichenized imperfect fungus). Non-lichenized fungi samples were grown during 3-10 days on a thin layer of PDA (39g/l) and OMA at 25°C. Samples of green algae and lichenized and non-lichenized fungi were fixed with 3% paraformaldehyde and 3% glutaraldehyde mixed in 0.1 M sodium cacodylate buffer pH 6.3. After 2 h, tissue plugs were cut from different sites of the colonies and postfixed in 1% osmium tetraoxide. The samples were dehydrated in a series of graded ethanol, pure propilene oxide or acetone and finally embedded in several resins, viz. Spurr's and Epon. Thick sections of resin-embedded specimens were treated with 2% paraphenylenediamine and the thin sections were contrasted with uranyl acetate and lead citrate. Specimens were examined in light microscope and Hitachi H-7000 transmission electron microscope.

Analysis of the micrographs revealed 6 nuclear phenotypes (NPH). The NPH 1 is characterized by the absence of nuclear pore complex and the presence of very few membrane fragments which are disposed either in linear or U shape. The

genetic material is found dispersed and associated directly with cytoplasmic ribosomes. NPH 2, shows a nucleus having one to four wide apertures and irregular ellipsoidal shape. The apertures lack a diaphragm and have a variable diameter between 160-1560 nm. In the interior of the nucleus of *C. calycantha* an elongated nucleolus enclosing an abundant euchromatin was observed. *Rhizoctonia solani* showed a nucleolus not separated from the cytosol, and ribosomes were seen associated to the genetic material in the interior of the nuclear region. NPH 3 with smaller apertures was present in *C. calycantha*. This phenotype shows simultaneously narrow (ca. 50 nm) and wide (ca. 400 nm) apertures and the genetic material is partially or not separated from ribosomes and the cytosol. Nuclear pores are unfrequently observed. This species has shown even other phenotypes (NPH 4) which has nuclei with narrow apertures (50-100 nm diam.); the DNA is partially separated from the cytosol and nuclear pores are rarely observed. NPH 5 showed the simultaneous presence of nuclear apertures (38-80 nm) and pores. Finally, NPH 6 shows the typical characteristics of the nuclear envelopes of upper eukaryotes or vertebrates, where the nuclear envelope has pores only. In some species such as in *Fusarium* sp. and *R. solani* nuclei are found lacking any evidence of nuclear envelope or membrane around the genetic material. In summary, the apertures found in the studied cells revealed approximate dimensions between 30 and 1570 nm in diameter and between 15 and 56 nm in length. Evidently these sizes are larger than those of the pores derived from upper eucaryotic cells, viz. 9-nm diam. and 15 nm long (Akey & Radermacher 1993, Ohno et al. 1998).

Molecules as long as mature cytosolic ribosomes cannot diffuse through the 9-nm channels, but can freely diffuse to the interior of the nucleus through such apertures. These apertures can facilitate nuclear export of newly made ribosomal subunits or import of large molecules (e.g. DNA and RNA polymerases) in less time.

Akey, C. W. and Radermacher, M.: 1993, *J. Cell Biol.* **122**: 1-19.
Cedeño, L. and Palacios-Prü, E.: 1990, *Turrialba* **40**: 356-367.
Cedeño, L. and Palacios-Prü, E.: 1992, *Acta Científica Venezolana* **43**: 178-189.
Cedeño, L. and Palacios-Prü, E.: 1996, *Acta Científica Venezolana* **47**: 24-29.
Gorlich, D.: 1997, *Curr. Opin. Cell Biol.* **9**: 412-419.
Nigg, E. A.: 1997, *Nature* **386**: 779-787.
Ohno, M., Fornerod, M. and Mattaj, L. W.: 1998, *Cell* **92**: 327-336.
Pante, N. and Aebi, U.: 1994, *J. Struct. Biol.* **113**: 179-189.

P3.21

COMPARATIVE BIOCHEMISTRY OF CO_2 FIXATION AND THE EVOLUTION OF AUTOTROPHY

Juli Peretó[1], Ana María Velasco[2], Arturo Becerra[2], and Antonio Lazcano[2]
[1]Departament de Bioquímica i Biologia Molecular, Universitat de València, E-46100 Burjassot (Spain); [2]Facultad de Ciencias, UNAM, Apdo. Postal 70-407, 04510 México DF (México)

Carbon dioxide fixation is a polyphyletic trait that has evolved in widely separated prokaryotic branches. The three principal CO_2-assimilation pathways are (a) the reductive pentose-phosphate cycle, i.e., the Calvin-Benson cycle; (b) the reductive citric acid (or Arnon) cycle; and (c) the net synthesis of acetyl-CoA from CO/CO_2, or Wood pathway. Sequence analysis and the comparative biochemistry of these routes suggest that all of them were shaped to a considerable extent by the evolutionary recruitment of enzymes. Molecular phylogenetic trees show that the Calvin-Benson cycle was a relatively late development in the (eu)bacterial branch, suggesting that some form(s) of carbon assimilation may have been operative before chlorophyll-based photosynthesis. On the other hand, the ample phylogenetic distribution of both the Arnon and the Wood pathways does not allow us to infer which of them is older. However, different lines of evidence, including experimental reports on the NiS/FeS-mediated C-C bond formation from CO and CH_3SH (Huber and Wächtershäuser 1997) are used to argue that the first CO_2-fixation route may have been a semi-enzymatic Wood-like pathway.

Huber, C. and Wächtershäuser, G.: 1997, Science **276**, 245-247

P3.23

MODEL FOR EMBRYONIC tRNAs AS: 1) CATALYSTS FOR PREBIOTIC AMINO ACIDS SYNTHESES VIA *IN SITU* TRANSFORMATIONS OF ONE LEFT-HANDED COMMON ANCESTOR, NAMELY L-PHOSPHOSERINE 2) PRIMITIVE TRANSLATION APPARATUS

Sylvain SMADJA
CSEOL.
corresp. address : 10751 Rose Ave. # 129, Los Angeles, CA 90034

One initial single chiral event might have been responsible for the creation of only right-handed sugars and consequently for the formation of only left-handed α amino acids.
This singular event might have been the early synthesis of glyceraldehyde in the pure right-handed form (process involving a ' surface catalysis by enantiomorphous mineral crystals', a ' sterospecific autocatalysis' or some physical effect? ...)
Such a hypothetical exclusive creation of D-glyceraldehyde would have had the following consequences:

-This triose could have been the direct precursor of a few significant sugars such as D-ribose and D-glucose.

- D-glyceraldehyde could have been converted into right-handed 2,3-bisphosphoglycerate (D-BPG).
A SN2 reaction of ammonia with D-BPG would have led to the creation of one left-handed α amino acid, namely L-phosphoserine.

- L-phosphoserine would then have been the common ancestor of a whole new class of left-handed α amino acids.
(The above suggestion, that the building units of proteins might have derived from a common ancestor, could help in explaining why each of these entities exhibits the α amino acid specific skeleton and it could also provide some answers of how Nature was able to achieve the chiral purity of such left-handed compounds.)

-Transformations of the carbon skeleton of L- phosphoserine entities could have taken place while such compounds were part of embryonic tRNAs. (Some authors, such as Wong and Danchin, had championed such an idea of prebiotic' cotranslational modifications'. According to them, such ' homeotopic transformations'might not have implicated only one common ancestor, but a few precursor amino acids.)

P3.23 continued

Embryonic tRNAs could have started as branched RNA structures. Such postulated complexes might have exhibited diverse clefts and cavities of different size and shape. Small and large molecules that would have been entrapped in such RNA pockets would have been able to react within themselves and with the host structures in various ways. In such a context bases could have been modified and the carbon skeleton of L-phosphoserine could have been more or less transformed.(In other words, it is suggested that primitive metabolisms could have occurred in the confines of such primordial RNA complexes.)
In the proposed embryonic tRNAs, L-phosphoserine moieties (already attached to the end of primal CCA chains) would have been directly *facing* some specific trinucleotides. Such triplets that might have been located at the' immediate proximity' to the L-phosphoserine residues would have been involved in the conversion of the common ancestor to new left-handed α amino acids.The side chain characteristics of each newly created amino acid would have mostly depended on the features (H bonding opportunities, hydrophobicity, bulkiness...) of each *facing* triplet.
At this point the author is suggesting a scenario by which embryonic tRNAs, carrying newly synthesized amino acids, could have evolved in such a way that the *facing* trinucleotides would have become the anticodonic triplets. Consequently it is argued here that the genetic anticode might have originated at the same time that the amino acids were being built. The 'lock and key' relationship that seems to exist between anticodons and cognate amino acids (Shimizu) might have developed when amino acids were presumably created in respective anticodonic pockets.
Finally each tRNA would have acted as the central unit in what would become the translation machinery.

Danchin, A. :1989, *Prog. Biophys. molec. Bio.* **54**,81.
Shimizu, M. : 1982, *J. Mol. Evol.* **18**, 297.
Wong, J. T. : 1975, *Proc. Natl. Acad. Sci. USA*, **72**, 1909.

P3.24

A GENETIC ALGORITHM FOR OLIGONUCLEOTIDE LIGATION

L. J. Sparvero and David A. Usher, Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853-1301

We have measured the rate of 2',5' internucleotide bond formation and cleavage in double-stranded RNA, using a carefully designed set of specific sequence oligomers, to compare with (1) our own work on homooligomers, and (2) the work of Eschenmoser (Bolli *et al*, 1997) on p-RNA.

The specific set of oligomers (a 20-mer, plus the 8-mer and 12-mer that are complementary to it) was chosen by means of a genetic algorithm, implemented in a computer, wherein natural selection (mutation and selective replication) was allowed to operate on a set of initially random 20–mer oligomer sequences. The genetic algorithm included point mutations, random crossovers, and cyclically permuted splice operations. Zuker's method (Zuker and Sankoff, 1984) for finding the secondary structure of a single strand of RNA was modified by introducing the improved energy parameters of Turner (Turner *et al*, 1988), and was then adapted to evaluate the fitness of each set of three oligonucleotides.The three oligomers that were selected for experimental study were chosen in part because they were expected to bind strongly to one another.

The ligation rate constant for the set of specific sequence oligomers showed a several-fold enhancement over that obtained for the ligation of homooligomers on a homopolymer under the same conditions of buffer and temperature.

Bolli, M., Micura, R., and Eschenmoser, A. :1997, Chemistry and Biology **4**, 309.
Fontana, W., and Schuster, P. :1998, Science, **280**, 1451.
Turner, D. H., Sugimoto, N., Freier, S. M. :1988, Ann. Rev. Biophys. Chem., **17**, 167.
Zuker, M., Sankoff, D. :1984, Soc. Math. Bio., 591.

P3.25

PORPHYRIN-PEPTIDE COMPLEXES IN ORIGIN AND EVOLUTION OF PHOTOSYNTHETIC REACTION CENTERS

Taisiya A. Telegina, Michael P. Kolesnikov , and Yulia L. Vechtomova

A.N. Bach Institute of Biochemistry, Russian Academy of Sciences. Moscow, Russia. E-mail <inbio@glas.apc.org>.

Solar radiation is the major source of energy and a selection factor both for abiotic and biotic evolution. The structure of solar spectrum has determined the structure of key substances passed through the evolution from its prebiological period to the biological one. The substances with conjugated double bonds such as N-containing heterocycles (pyrroles, imidazoles, purines, pyrimidines, indoles, etc.) were formed and selected in photochemical processes. Being UV resistant, these substances were photoactive in UV-region, and after evolution they adapted to use the visible light owing to the increase of size of molecules, their complexing, *etc.*

Solar spectrum governed the formation and evolution of pigment systems and of the photosynthetic apparatus as a whole. It was shown, that both porphyrins and peptides were formed under the UV-radiation. The selection of Mg-porphyrin complexes in the process of evolution was likely to occur with the participation of light.

Our studies showed that under the effect of light, Mg^{2+} incorporated into protoporphyrin IX (PP-IX) more actively than other metals (Zn^{2+}, Cd^{2+}, Fe^{3+}). It was shown, that the formation of complexes of peptides (and proteinoids as well) with the evolutionary precursors of chlorophyll (protoporphyrin IX, *etc.*) increased photochemical activity of these pigments. We suggested the histidine-containing peptides could favor the development of photosynthetic RC and ETC due to the histidine's capacity to donate and accept electrons. The PP-IX complexes with histidine-containing proteinoids demonstrated a higher photocatalytic activity in photooxidation of glycolic acid with O_2 and in the methyl red photoreduction by ascorbate as compared to the PP-IX itself, whereas its complexes with the histidine-free proteinoids were less active in these reactions than non-bound porphyrin molecules. The formation of pigment-protein complexes was a light-dependent process: the irradiation of PP-IX+Mg^{2+}+histidyl-leucine mixture led to these complexes formation. Investigation of photosensitizing activity of these complexes showed, that their activity in methyl red photoreduction with ascorbate was higher in presence of [PP-IX-Mg^{2+}-his-leu] complex, than with a mixture of PP-IX, Mg^{2+} and his-leu not subjected to irradiation. The results are discussed in connection with the origin of photosynthetic RC.

P3.26

TEMPLATE-DIRECTED NUCLEOTIDE OLIGOMERIZATIONS ON METAL-ION SUBSTITUTED HYDROXYAPATITES

Herbert van de Voort, Johannes Visscher and Alan W. Schwartz
Evolutionary Biology Research Group, Faculty of Science, University of Nijmegen, Postbus 9010, 6500 GL Nijmegen, The Netherlands.

Template-directed oligomerization is a well-known model for a possible prebiotic process. In addition, certain divalent metal ions are noted to be efficient catalysts for oligonucleotide synthesis and could thus have played an important role in the development of a non-enzymatic, self-replicating system. On the prebiotic Earth, however, catalytically active divalent metal ions such as Pb(II) and Zn(II) would occur in the prebiotic ocean in trace concentrations only. This means that for oligomerization to have been of any real importance in the origin of life, there would have to have been a mechanism which could locally increase the concentration of both the reactive species and the geochemically sparse but catalytically interesting metal ions.

Experiments have shown it to be possible to accumulate divalent metal ions onto hydroxyapatite from very dilute solutions. Oligomerization reactions performed with prepared hydroxyapatites containing Pb(II) or Zn(II), showed an enhancement and selectivity of oligomer formation comparable to the reactions with the free metal ions in solution.

By combining the sorption properties of hydroxyapatite with the catalytic effects of Pb(II) and Zn(II) ions, it seems possible to obtain an improved model of prebiotic oligonucleotide synthesis.

P3.27

TEMPLATE-DIRECTED SYNTHESIS BY A MEMBRANE-ENCAPSULATED POLYMERASE: A MODEL PROTOCELL

Wenonah Vercoutere, Pierre-Alain Monnard, David Deamer
Chemistry, UCSC, Santa Cruz CA, USA 95060

One conjecture for the origin of life is that simple self-assembled replicating systems were the precursors to the unicellular organisms that appeared more than 3.5 billion years ago. These protocells are likely to have included a recurring polymerization reaction entrapped within a defined membrane compartment. Access to nutrients and chemical energy would have been through passive diffusion. Our aim is to model such a protocellular system using a nucleic acid-replicating enzyme, T7 RNA polymerase, and a DNA template encapsulated within liposomes.

Encapsulation of the template and enzyme was carried out by a dehydration-rehydration method (Shew and Deamer, 1983).

The permeability of the phosphatidylcholine membranes to ionic solutes, as well as to the negatively charged nucleotides, is facilitated by transient defects (Paula et al., 1996), and can be maximized at the phase transition temperature of the liposome bilayers (Kanehisa and Tsong, 1978). Therefore the liposome suspensions with encapsulated template and enzyme were subjected to repetitive temperature cycling between the optimal temperatures for diffusion of externally added substrate molecules and for the enzymatic reaction.

Our results indicate that RNA was synthesized within some liposomes. These observations demonstrate that the passive diffusion of large ionic substrate molecules (NTPs) across lipid bilayers at their phase-transition temperature may be adequate to supply an encapsulated template-dependent polymerase. Therefore, primitive protocellular systems could have relied on passive diffusion to take up nutrients from their environment.

Kanehisa, M.I. and Tsong, T.Y.: 1978, JACS **100**, 424-432.
Paula, S., Volkov, A.G., Van Hoek, A.N., Haines, T.H. and Deamer, D.W.: 1996, Biophys. J. **70**, 339-348.
Shew, R.L. and Deamer, D.W.: 1985, Biochim. Biophys. Acta **816**, 1-8.

i4.1

COMPARATIVE MICROBIAL GENOMICS: INSIGHTS ON PHYSIOLOGY AND EVOLUTION

Karen E. Nelson, Ph.D.
The Institute for Genomic Research, Rockville, MD, USA 20850

Microbial genome-sequencing efforts at TIGR between 1995 and the present have produced complete genome sequences for nine organisms: *Haemophilus influenzae*, *Mycoplasma genitalium*, *Methanococcus jannaschii*, *Helicobacter pylori*, *Archaeoglobus fulgidus*, *Borrelia burgdorferi*, *Treponema pallidum*, *Thermotoga maritima*, and *Deinococcus radiodurans*. In addition, 18 other microbial genome projects are either in progress or soon to be started, with an estimated completion date for all work of 2001. In total, the microbial genome sequencing projects at TIGR represent a total of nearly 75 million base pairs of DNA and an estimated 70,000 microbial genes. The information generated in these projects is available from the TIGR Microbial Database that can be accessed via the World Wide Web at www.tigr.org. In addition, work in other laboratories around the world will generate an additional ~75 million base pairs of new microbial genome sequence data during the next 2-3 years.

The availability of genome sequence information from a range of microbial species allows for detailed comparative genomic analysis. However, with approximately one-half of all predicted coding sequences from each organism unassigned a putative biological function, there is considerable microbial biology yet to be elucidated. In addition, approximately one-quarter of the predicted coding sequences from each genome appear to be unique. The available sequence data also provides an opportunity for a more comprehensive understanding of microbial evolution using overall genome content rather than single gene analysis. The *Thermotoga maritima* genome sequence in particular reveals a tremendous degree of lateral gene, and casts doubt on the validity of small subunit rRNA phylogeny as a reliable measure of whole species evolution.

i4.2

HOW FAR BACK CAN WE SEE THROUGH GENOME COMPARISON

Eugene V. Koonin
National Center for Biotechnology Information, NIH, Bethesda, MD 20894, USA

Computer analysis of complete genomes of unicellular organisms shows that protein sequences are in general highly conserved in evolution, with at least 70% of them containing ancient conserved regions. This allows us to delineate families of orthologs across a wide phylogenetic range and in many cases, predict protein functions with reasonable confidence. Examination of the 'phylogenetic pattern' for these orthologous families shows that only about 100 families, most of which include components of the translation machinery, are universally conserved in all sequenced genomes. Thus horizontal gene transfer and lineage-specific gene loss are not inconsequential evolutionary quirks but rather prevailing forces of evolution, at least in the prokaryotic world. Horizontal transfer and lineage-specific loss of entire genes are complemented by numerous intragenic recombination events that manifest in domain rearrangement at the protein level.

Examination of phylogenetic patterns for families of orthologous proteins also results in more specific conclusions some of which may have far-reaching consequences. In particular, it is now clear that the basic DNA replication machineries (that is, the replicative DNA polymerases, primases, helicases, and several other proteins) in bacteria and in archaea/eukaryotes are *not* orthologous and may have evolved independently. This leads to a hypothesis that the common ancestor of all extant cellular life forms (the cenancestor) did not possess a modern-type, DNA-based replication and expression system although it did encode advanced translation and transcription machineries and a considerable repertoire of metabolic enzymes. Instead of a dsDNA genome, the cenancestor might have had a mixed system of small RNA and DNA genetic elements that were interconverted via cycles of transcription and reverse transcription. This model seems to account for both universal and distinct components of the DNA replication machinery in bacteria and archaea-eukaryotes. Comparison of proteins involved in genome replication and expression using sensitive methods for sequence analysis allows a tentative reconstruction of some of the broad specificity enzymes that could have functioned in the cenancestor, for example an enzyme that could combine properties of a primase, a topoisomerase and a nuclease.

i4.3

REGROWING THE TREE OF LIFE

Russell F. Doolittle
Center for Molecular Genetics, University of California, San Diego,
La Jolla, California 92093-0634, USA

The availability of complete genome sequences from a score of microorganisms--especially Bacteria (B) and Archaea (R)--has forced a reconsideration of how the three principal domains of extant life are related. Until recently, the most widely accepted model of the "Tree of Life" postulated that the Eukarya (K) constitute a sister group of the Archaea, as symbolized by the expression B(RK). This arrangement was based on an analysis of sequence relationships for some ubiquitous paralog pairs, the engendering duplications of which were thought to have occurred in advance of the divergences leading to the three groups of organisms. The new data cast doubt on that relationship, the majority of gene product phylogenies exhibiting the pattern R(BK), the proteins of Bacteria being more similar to those of Eukarya than are those from Archaea. The possibility exists that many of these proteins reflect the acquisition of genes from an endosymbiont by a eukaryotic host.

There is another group of gene products, however, which is described by the relationship (RB)K. Some of these appear to be the result of horizontal transfers between Archaea and Bacteria. A third group of gene products does indeed exhibit the pattern B(RK) as had been earlier supposed on the basis of the paralog test.

How are we to reconcile these apparently conflicting gene trees? There are scenarios that afford a self-consistent and coherent series of events and that are also in accord with timetables based on protein clocks. Briefly put, the lineage leading to Archaea and Bacteria would have separated from that leading to Eukarya at a very early point in evolution, as has been suggested by others, and before the divergence of Bacteria and Archaea. Subsequently, bacterial genes would have found their way into the eukaryotic genome as a result of transfer from bacterial endosymbionts.

i4.3 continued

These gene products would have the R(BK) arrangement. Similarly, archaeal genes could also have been acquired by eukaryotes by transfer from archaeal endosymbionts. These gene products would exhibit the B(RK) pattern. Finally, some eukaryotic genes are descendants of the original host divergence, including those for the cytoskeleton. These have the arrangement (BR)K. Characterization of extant eukaryotes which contain both bacterial and archaeal endosymbionts may shed more light on the situation.

c4.4

ON THE STABILIZATION OF THE GENETIC CODE AND FUNCTIONS IN THE ORIGIN OF LIFE.

CLAS BLOMBERG,
Department of Physics, Royal Institute of Technology
10044 Stockholm, Sweden

An outstanding problem for the origin of life is to understand how processes of life could have been stabilized. Catalyzed self-replicating processes and eventually a template-driven protein synthesis are essential but could never have evolved into a real, living organism unless a large variety of functions had emerged with possibilities to stabilize the processes through feedback loops. It is also essential that the variety of functions must have occurred more or less spontaneously. A simpler system is unlikely to develop into such a large variety only by evolutionary laws.

We will discuss this problem starting from general ideas, exemplified by mathematical models, on destabilizing possibilities and what should have been necessary for stabilization. An important aim is to get general statements on what kind of feed back processes that were necessary, and ideas on how these could have been accomplished. An important question concerns probabilities. A general principle is that no mechanism can occur in a simple way because it is needed: as all functions, it must occur by selection from a randomly created manifold.

A particular example that will be discussed is the genetic code. According to our general idea, the code must have been stabilized by feedback mechanisms: codes were stabilized by providing enzymes that could control the mechanisms by which they were formed. This principle suggests a kind of frozen accident scenario, but should be reconciled together with other views on the origin of the genetic code to provide something that to some parts is systematic, to some part accidental.

Blomberg, C: 1997, J. theor. Biol. 187, 541-554.

c4.5

HAS NATURAL SELECTION SHAPED THE GENETIC CODE?

Stephen J. Freeland[†], Robin D. Knight[†], Laurence D. Hurst[¶] & Laura F. Landweber[†]

[†] Princeton University; [¶] University of Bath, UK

Why does the genetic code take the precise form that it does? We now know of an abundance of naturally occurring variant genetic codes, indicating that codon assignments within the standard genetic code are unlikely to represent the 'frozen accident' proposed by Crick (1968). Three classes of explanation have subsequently emerged to explain its structure (Knight *et al.*, 1999). *Error minimisation* hypotheses propose that the code is adaptive, with codon assignments arranged to minimise the phenotypic impact of genetic errors such as point mutation and mistranslation. *Biosynthetic* hypotheses propose that today's genetic code evolved from a simpler ancestral form (encoding fewer amino acids with greater redundancy): new amino acids, produced as the by-products of early metabolism, were incorporated into the code by capturing a subset of the codons previously assigned to their biosynthetic precursors. Finally *stereochemical* hypotheses propose that the formation of the code (and therefore of protein based life) resulted from intrinsic chemical affinities between amino acids and their corresponding codons.

I present evidence for the error minimisation hypothesis of code evolution. First I demonstrate a simple comparative model by which the standard code may be compared with randomly generated plausible alternatives, in terms of the average effect of errors (single point mutations). This basic model indicates the probability of evolving a code as efficient as that used by nature is of the order 0.0001 (Freeland and Hurst 1998). Increasing the sophistication of the model, by incorporating known biases in the patterns of biological errors, reduces this probability by up to 2 further orders of magnitude. Next I show that this evidence for an adaptive code structure cannot be dismissed as an artefact of a biosynthetic explanation for code structure. Finally I incorporate recent evidence for a stereochemical origin for the code (Knight and Landweber 1998), and suggest a possible synthesis of the 3 hypotheses (Knight *et al.* 1999), placing adaptive evidence into a broad model of code evolution.

Ardell, D.H. :1998, J. Mol. Evol. **47**, 1.
Crick, F. H. C. :1968, J. Mol. Biol. **38**, 367.
Freeland, S.J. & Hurst, L.D. :1998, J. Mol. Evol. **47**, 238.
Knight, R. D., Freeland S. J. & Landweber L.F. :1999 TiBS, *In press.*
Knight, R. D. & Landweber, L. F. :1998, Chemsitry and Biology **5**, R215.

c4.5 continued

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Stephen J. Freeland[†], Robin D. Knight[†], Laurence D. Hurst[¶] & Laura F. Landweber[†]

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Ardell, D.H. :1998, J. Mol. Evol. **47**, 1.
Crick, F. H. C. :1968, J. Mol. Biol. **38**, 367.
Freeland, S.J. & Hurst, L.D. :1998, J. Mol. Evol. **47**, 238.
Knight, R. D., Freeland S. J. & Landweber L.F. :1999 TiBS, *In press.*
Knight, R. D. & Landweber, L. F. :1998, Chemsitry and Biology **5**, R215.

c4.6

COMPARATIVE GENOMICS: PRODUCTS OF THE MOST CONSERVED PROTEIN-ENCODING GENES SYNTHESIZE, DEGRADE, OR INTERACT WITH RNA

F. Tekaia[1], B. Dujon[1], and A. Lazcano[2]

[1]Unité de Génétique Moleculaire des Levures (CNRS URA1300)
Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris, FRANCE
[2]Facultad de Ciencias, UNAM/ Apdo. Postal 70-407
Cd. Universitaria, 04510 México D. F., MEXICO

A systematic search for the most highly conserved protein-encoding sequences in the cellular annotated genomes available as of December 1998 was perfomed. Intra- and intergenome comparisons were performed using *blastp* version 1.4.8 or *tblastn*, each required. Each predicted open reading frame (ORF) product of a given organism served sucessively as a query sequence against the entire databse of that same species, and against the database of every other genome considered in this analysis. To account for the differences in size and comlexity, the significance of *blatp* probability scores were determined for each genome using sets of random sequences which were generated with sizes and compositions equal to each genome studied. Among the most conserved sequences detected in this analysis are those of genes encoding proteins involved in gene expression and RNA metabolism. Transcription and translation genes are as conserved ad DEAD-type RNA helicase and enolase genes, which form part of the RNA degradosome. Conserved sequences related to metabolism include those encoding putative PRPP synthase, thioredoxin, and serine hydromethyltransferase, which are all involved in nucleotide biosynthesis. The conservation of an ancient core of proteins which synthesize or interact with RNA that we have detected are probably best understood in terms of an early evolutionary period during which RNA may have had a more prominent role in biological processes. Nevertheless, the presence of metabolic genes involved in deoxyribonucleotde biosynthesis suggest that the last common ancestor of all living beings already had developed a DNA genome.

c4.7

EVOLUTION AND CHARACTERIZATION OF AN UNNATURAL ORGANISM

Jamie Bacher and Andrew D. Ellington
Department of Chemistry and Biochemistry
University of Texas at Austin, Austin, TX, USA, 78712

In order to explore whether unnatural amino acids could be introduced into proteins *in vivo* and whether the chemistry of an entire organism might thereby be manipulated, we have attempted to introduce an unnatural amino acid, 4-fluorotryptophan (4fW), in place of a natural amino acid, tryptophan, throughout the entire proteome of the bacteria *Escherichia coli*. After 3,500 hours of continuous evolution of a tryptophan auxotroph, we have derived an 'unColi' that can grow solely in the presence of 4fW. Amino acid and mass spectral analyses confirm the replacement of the natural amino acid with the unnatural amino acid. In addition, we have characterized mutations that facilitate 4fW incorporation. The number and identity of these mutations suggests it may be possible for organisms to readily change even their most basic biochemistries.

Just as the discovery of non-canonical triplet codons in mitochondria suggested that the genetic code might not be sacrosanct, the ability to introduce unnatural amino acids into organismal proteomes suggests that the 20 amino acids themselves may not be unalterably fixed in evolutionary history. Ultimately, these findings have two major implications: first, it may be possible not only to alter the chemistries and biochemistries available to proteins, but also to entire organisms. Since it is organisms, not proteins, that are most directly selected and evolved, it may be possible to provide an organism with novel chemistries in the form of unnatural amino acids, nucleotides, or cofactors, and then use natural selection to generate unanticipated biochemistries and phenotypes. Second, our explorations of terrestrial biochemistry may not have completely prepared us for the types of biochemistries that will be found on other planets. By evolving and characterizing unnatural organisms we may be able to better identify the boundary conditions for living systems, and to understand what chemical variables most dramatically affect the definition of life itself.

P4.1
WHAT IS THE FIRST LIVING BEING?

Anatoly D. Altstein
Institute of Gene Biology, 117334, Moscow, Russia

The main aim of investigations on the origin of life are to understand and ground theoretically and experimentally how the first and most primitive living being (FLB) emerges in the prebiotic world, self-reproducing and evolving to the modern cell. That is why it is important to differentiate FLB from abiotic systems such as liposomes, microspheres, coacervates, replicating oligonucleotides and others. The main difference is presence of a genetic system (GS) in FLB, i.e. a molecular system that ensures self-reproduction and evolution on the principle "heredity-variability-natural selection". Life without GS is nonsense. Hence the transition point between the prebiotic and the alive world is emergence of the self-reproducing and evolving GS. Compartmentalization and metabolism are also necessary for arising and existing of GS.

Two models of FLB are possible: (1) FLB is the most primitive protocells (unity of GS, membrane and metabolism); (2) FLB is a virus-like (protoviroid) GS that uses abiotic compartments and abiotic metabolism. The GS of the most primitive protocell must be too complicated (10-15 genes) to arise in the prebiotic environment. That is why the 2nd model is more preferable.

Earlier I proposed the progene hypothesis to explain arising and replication of the minimal GS containing of one gene (single stranded polynucleotide) and its polypeptide product – processive polymerase (see Altstein, 1992a, 1992b). On the hypotesis both components of GS are synthesised not from mononucleotides and free amino acids but from preformed progenes – trinucleotides acylated by a non-occasional amino acid on 3'-γ-phosphate (NpNpNpppp-Aa). The hypothesis explains a mechanism of progene formation from dinucleotides (NpNp) and aminoacyl nucleotides (Nppp-Aa) on the basis of a specific interaction between the Aa's and dinucleotides. It explains also how GS originates from the progenes as an extremely rare event and than replicates and translates using the progenes as substrates and amino acid adaptors for synthesis of the polynucletide and the polypeptide. The hypothesis allows to explain the origin of the first primitive physico-chemical group genetic code (4 groups of amino acids could be coded in according with

P4.1 continued

2^{nd} nucleotides of the progenes; this conclusion was made on the basis of stereochemical analysis results). It is suggested that the progenes are synthesised in prebiotic liposomes that in moment of their formation (*in statu nascendi*) capture microcristals of activated nucleotides and amino acyl nucleotides – product of the abiotic metabolism. Nothing except the progenes and the conditions for their synthesis are needed for arising and replication of GS. Such a GS can be considered as FLB – *Protoviroidum primum* with the minimal genome . It had polynucleotide (preferablly DNA)-protein nature and reproduces using abiotic sources of activated metabolites and migrating from one liposome *in statu nascendi* to another one. *P. primum* can't be pure polynucleotide and lead to RNA world formation because ribozymes apparently can't be processive polymerases. That is why I suggest that the RNA world of living beings never existed. I propose the following scheme of origin and evolution of life: prebiotic world →protoviroid world (polynucleotide-protein)→protocell world (stormy evolution of genetic and biochemical mechanisms)→modern cell world.

Altstein, A.D. : 1992a, Seminars in Virology **3,** 409
Altstein, A.D.: 1992b, Frontiers of Life, Edition Frontieres, Paris, p.425

P4.2

PHYLOGENETIC DISTRIBUTION OF SIMPLE SEQUENCES: INSIGHTS FROM COMPARATIVE GENOMICS

Arturo Becerra and Antonio Lazcano
Facultad de Ciencias, UNAM/Apdo. Postal 70-407
Cd. Universitaria, 045210 México D. F., MEXICO
E-mail: alar@hp.fciencias.unam.mx

Simple sequences are segments or regions of protein and nucleic acid sequences which are biased in residue composition, and typically contain repetitive DNA. Homopolymeric tracts and tandem arrays of multiple short repeat motifs have their origin in slipped-strand mispairing during DNA replication, and due to their hypermutable character are recognized as a major source of random phenotypic variation, especially among prokaryotic pathogens (Moxon *et al.*, 1994; Hancock, 1996). In order to obtain insights on the phylogenetic distribution of simple sequences and their biological properties, we have used the SEG program (Wootton and Federhen, 1993), with a trigger window length (W) value of W=30, trigger complexity (K1) of K1=2.8, and local compositional complexity K2 equal to 3.0, to analyze 17 complete cellular genomes available in public databases as of February 1999. Our results indicate that simple sequences (a) have a wide phylogenetic distribution, i.e., their appearance is older than microbial pathogens; (b) are not restricted to a unique class of enzymes, but are present in catalytic and structural proteins involved in a wide spectrum biological functions; (c) are be found both at the C- and N-ends of proteins; (d) there is a compositional bias in simple sequences, which tend to be rich in alanine, leucine, lysine, serine and glutamine, while histidine, tryptophan, and cisteine are underrepresented; and (e) homopolymeric tracts in hyperthermophiles are enriched in glutamine. The role of simple sequences in protein evolution will be discussed.

Hanock, J. M.: 1996, *Nature Genetics* **14**, 14
Moxon, E. R., Rainey, P. B., Nowak, M. A., and Lenski, R. E.: 1994. *Curr. Biol.* **4**, 24
Wootton, J. C.and Federhen, S.:1993, *Comp. Chem.* **17**, 149

P4.3

DID THE LAST COMMON ANCESTOR HAD A DNA GENOME?

A. Becerra[1], J. I. Leguina[2], L. Delaye[1], and A. Lazcano[1]

[1]Facultad de Ciencias, UNAM/ Apdo. Postal 70-407
Cd. Universitaria, 04510 México D. F., MEXICO
[2]Protein Design Group, Centro Nacional de Biotecnología-CSIC
Cantoblanco, 02849 Madrid, SPAIN

One of the problems surrounding the nature of the last common ancestor (i.e., LCA or cenancestor), is wether it possesed an RNA genome (Mushegian and Koonin, 1996), or whether it was already endowed with a DNA genome (Becerra *et al.*, 1997). As part of an attempt to develop a more detaled picture of the cenancestor, we have analised all the available sequences of the proteins involved in DNA metabolism available in the public databases that are known to be present in the three primary urkingdoms, i.e., the Bacteria, the Archaea, and the Eucarya. The search was conducted by comparing the sequences across the three domains using BLASTP algorithm, and constructing multiple aligments using the MACAW program. Our results suggest that the LCA had genes directly involved in DNA metabolism. These genes include those involved in DNA replication (DNA primase, RecQ protein, DNA polymerase B, and type I and II topoisomerases), DNA repair (RAD genes, photolysases, uracil DNA glycosylase), and in deoxyribonucleotide biosynthesis, including ribonucleotide reductase, thymydilate synthase, thioredoxin, and serine hydroxymethyltransferase. The conservation of the latter is surprisingly high. The possibility of horizontal gene transfer notwithstanding, these results strongly suggest that the cenancestor was much akin to extant prokaryotes in the basic details of genetic information storage mechanisms.

Becerra *et al.*: 1997, *J. Mol. Evol.* **45**, 115
Mushegian, A. R. and Koonin, V. E.: 1996, *PNAS* **93**, 10268

P4.4

DID PROKARYOTIC DNA CONTENT EVOLVE BY A SERIES OF GENOME DOUBLINGS?

Amanda Castillo, Sara Islas, and Antonio Lazcano

Facultad de Ciencias, UNAM
Apdo. Postal 70-407
Cd. Universitaria, 045210 México D. F., MEXICO
E-mail: alar@hp.fciencias.unam.mx

Distribution of prokaryotic genome sizes and the position on the *E. coli* chromosomal map of pairs of related genes led to suggestions that genome sizes have evolved through a series of sequential large-scale genome duplications of ancestral chromosomes (Wallace and Morowitz 1973; Zipkas and Riley, 1975). In order to test this hypothesis, we have analyzed a database constructed from published reports of over 165 prokaryotic genomes whose size has been determined by pulse-field gradient electrophoresis. Although this database is likely to be biased and does not represents the full range of prokaryotic diversity, the different peaks we observe in the discontinous distribution of DNA content do not support the idea that several duplications involving first a hypothetical ancestral minigenome took place during evolutionary time.

Attempts to correlate genome size distribution with 16S rRNA-based phylogenies indicate that major gene losses like those of the mycoplasma and rickettsia are irreversible. When the genome size distribution was analyzed in relation with the oxygen-response of the different bacteria as reported in the literature (i.e., anaerobic, facultative, microaerophilic, and aerobic), it was found that in general obligate anaerobes and/or microaerophiles have genomes much more smaller than the aerobic bacteria, although there is considerable variation among the latter. Larger genomes are typical of free-living bacteria (i.e., myxobacteria and actinobacteria) with complex life cycles. Although the likelihood of large-scale, complete genome duplications cannot be outruled, the available data suggest that genome sizes are probably due to other processes, including gene elongation, duplication of genome segments of various lengths, slippage, and horizontal transfer.

Wallace, D.C. and Morowitz, H.J.: 1973, *Chromosoma* **40**: 121
Zipkas, D. and Riley, M.: 1975, *PNAS* **72**: 1354

P4.5

ANCIENT RNA-BINDING SITES: FROM THE CENANCESTOR TO THE THREE PRIMARY DOMAINS

Luis Delaye and Antonio Lazcano
Facultad de Ciencias, UNAM/ Apdo. Postal 70-407
Cd. Universitaria, 04510 México D. F., MEXICO
E-mail: alar@hp.fciencias.unam.mx

Comparisons of complete cellular genomes indicate that a set of genes whose products synthesize, degrade, or interact with RNA molecules are among the most highly sequences common to all living beings, and therefore may have been present in their last common ancestor, i.e., the cenancestor. These sequences include those encoding the two largest subunits of DNA-dependent RNA polymerases, DEAD-type RNA helicases, and ribosomal proteins, among others. In order to obtain insights on the evolution of sequences which may date from an early evolutionary period during which RNA may have played a genetic role prior to the emergence of DNA genomes, we have analyzed the conserved functional and structural sites of these highly conserved molecules. Special attention was given to RNA-binding sites, since these may be some of the oldest motifs in our dataset. The characteristics of these highly conserved amino acid stretches which are essential in RNA metabolism will be discussed.

P4.6

SPONTANEOUS REACTIONS IN METABOLISM: IMPLICATIONS FOR THE ORIGIN OF BIOSYNTHETIC PATHWAYS

J. I. Leguina[1], R. Gómez-Balderas[2], V. J. Aran[3], and Antonio Lazcano[4]

[1]Protein Design Group, CentroNacional de Biotecnología-CSIC/28049 Madrid, Spain
[2]Química Téorica, Facultad de Química, UNAM/México D. F., México
[3]Centro de Química Orgánica "M. Lora Tamayo", CSIC/Juan de la Cierva 3, 28006 Madrid, Spain
[4]Facultad de Ciencias, UNAM/ Apdo. Postal 70-407/Cd. Universitaria, 04510 México D. F., México

Most steps in biosynthetic pathways are mediated by enzymes, but some occur spontaneously. In otehr cases, the corresponding chemical change can be achieved under in laboratory conditions by altering the reaction conditions and reagents in the absence of the enzyme. These observations support the possibility that portions of biosynthetic routes resulted from non-enzymatic reactions, followed by the evolutionary acquisition of starter type enzymes. Characterization of these catalyst-free recations can therefore shed information on the possibility of semi-enzymatic pathways during the early stages of metabolic evolution once the DNA/protein world had appeared.

Here we will present an example of what may be one of these relict reaction, that forms part of proline anabolism, and which forms part of the glutamate family. During proline biosynthesis the reduction of the γ-carboxyl group yields L-glutamate semialdehyde, which undergoes an spontaneous cyclization reaction to yield Δ^1-proline-g-semilaldehide, a five-membered ring compound which is then reduced to yield proline. An exhaustive search of all kinds of spontaneous reactions found in different metabolic pathways has showed that their actual number is very small, but but some of them are part of ancient biosynthetic routes. Formation of an internal Schiff base, cyclic lactones, and lactams appear to be the principal types of non-enzymatic recations found in all living cells

P4.7

EVOLUTION OF LYSINE BIOSYNTHESES

A. M. Velasco[1], J. I. Leguina[2], and A. Lazcano[1]

[1]Facultad de Ciencias, UNAM/ Apdo. Postal 70-407
Cd. Universitaria, 04510 México D. F., MEXICO
[2]Protein Design Group, Centro Nacional de Biotecnología-CSIC
Cantoblanco, 02849 Madrid, SPAIN

Among the different biosynthetic pathways found in extant organisms, lysine biosyntehsis is peculiar because it has two different anabolic routes. One is the so-called diaminopimelic acid pathway (DAP), and the other the α-aminoadipic acid pathway (AAA). The DAP is present in (eu)bacteria, lower fungi, and plants (Ragan, 1989), as well as in methanogenic archaea (Bakhiet et al., 1984). The AAA pathway is used by higher fungi, euglenoids, and possibly some archaea as *Sulfolobus* (Ragan, 1989).

The fact that two anabolic pathways exists for lysine offers a good model to establish which of the different theories of metabolic evolution was operational in their development. Here we report the results of a deatiled analysis of the sequences of each of the enzymes involved in the two routes. We found that there is no apparent eovlutionary relationship between the pathways. Our results suggest that the DAP is related to arginine anabolism, since the products of the genes *lysC*, *asd*, and *dapE* genes from lysine biosynthesis are related to the *argB*, *argC*, and *argE* genes, respectively, whose products catalyze different steps in arginine anabolism. On the other hand, the AAA pathway is related to antibiotic biosynthetic pathways. These results suggest that the two routes for lysine biosynthesis were assembled via the patchwork mechanism (cf. Jensen, 1976). We also conclude that enzyme recuritment occurred both before and after the last common ancestor.

Bakhiet *et al.*: 1984, *Curr. Microbiol.* **10**, 195
Jensen, R. A.: 1976, *Annu. Rev. Microbiol.* **30**, 409
Ragan, T.: 1989, *in The Hierarchy of Life*, K. Bremer and H. Jornvall (eds), Elsevier, p. 145

P4.8

PHYLOGENETIC ANALYSIS OF THE ANCIENT PARALOGOUS MODULES *hisA 1, hisA2, hisF1*, AND *hisF2*

Ulises Iturbe[1], Renato Fani[2], and Antonio Lazcano[1]

[1]Facultad de Ciencias, UNAM/ Apdo. Postal 70-407
Cd. Universitaria, 045210 México D. F., MEXICO

[2]Dipartamento di Biologia Animale e Genetica/ Universitá degli Studi di Firenze/ Via Romana, 17, I-50125 Firenze, ITALY

The use of paralogous genes, i.e., sequences that have diverged not through speciation but after duplication events, has allowed the construction of rooted universal phylogenies, which indicate a sisterhood relationship between the Archaea and the Eucarya, and the recognition of the Bacteria as the oldest pehnotype. This result, which was achieved using the α and β hydrophilic subunits of F-type ATP synthases (Gogarten *et al.*, 1989) and the two elongation factors (EF-Tu and EF-G) that assist in protein biosynthesis (Iwabe *et al.*, 1989), has been confirmed using other sets of paralogous genes, which include those of aminoacyl-tRNA synthases, but not by phylogenetic trees based on metabolic genes such as *hisA* and *hisF* (Charlebois *et al.*, 1997).

In order to understand the molecular evolution of genes involved in histidine biosynthesis, we have analyzed the set of *hisA* and *hisF* genes and their homologues available in public databases as of February 1999. These sequences are known to be the outcome of gene elongation events that led first to the *hisA* gene from two ancestral modules, followed by a paralogous duplication that originated the *hisF* sequence (Fani et al., 1995). These elongation and duplication events appear to be irreversible and conserved in all known organisms. Results on the use of these sequences of these modules to construct rooted phylogenetic trees will be presented. We will also discuss the possibility that the *hisA* and *hisF* sequences have been homogenized by homologous recombination following the divergence of the three cell domains from the cenancestor.

Charlebois *et al.*: 1997, *J. Bacteriol.* **179**, 4429
Fani, R., Lio, P., and Lazcano, A.: 1995, *J. Mol. Evol.* **41**: 760
Gogarten *et al.*: 1989, *Proc. Natl. Acad. Sci. USA* **86**: 6661
Iwabe *et al.*: 1989, *Proc. Natl. Acad. Sci. USA* **86**: 9355

P4.9

EVOLUTION OF MINIMALIST FUNCTIONAL PROTEINS

J. D. Hirst
Department of Molecular Biology, TPC-6, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla CA, USA 92037

Genome sequencing efforts are generating a huge amount of data. Many of these data, such as each new protein sequence, is interesting in its own right. General features of genome data are also of great interest. For example, proteins operate under a number of constraints. They have to be stable. They have to adopt a unique native state. They have to function. They fold cooperatively. They have to be tolerant to mutation. How do these constraints influence evolution?

The earliest biopolymers may have been short heteropolymers. We investigate a minimalist model for proteins with binding pockets, called functional model proteins, based on a shifted-HP model (Chan & Dill, 1996) on a two-dimensional square lattice. In the HP model every sequence consists of only two types of residue: hydrophobic, H, and polar, P. Only nearest neighbor interactions are counted. Interactions between hydrophobic residues are favorable. In the standard HP model, all other interactions are zero. In the shifted-HP model, all other interactions are unfavorable. The repulsive interactions in the shifted-HP model provide a mechanism for creating binding pockets.

These model proteins are not maximally compact and contain an empty lattice site surrounded by at least three nearest neighbors, thus providing a binding pocket. Functional model proteins possess a unique native state, cooperative folding and tolerance to mutation. Due to the explicit functionality in these models (by design), we have been able to explore their fitness or evolutionary landscapes, as characterized by the size and distribution of homologous families and by the complexity of the inter-relatedness of the functional model proteins. Mindful of the caveats associated with minimalist models, functional model proteins should provide a useful means for exploring the constraints of maintaining structure and function on the evolution of proteins.

Chan, H. S. and Dill, K. A. :1996, Proteins **24**, 335.

P4.10

CLAY MINERALS AS A RESTING-PLACE OF GENETIC MATERIAL IN PRIMEVAL HABITATS

Marco Franchi and Enzo Gallori
Department of Animal Biology and Genetics, University of Florence
Via Romana, 17 - 50125 - Florence, Italy
E-mail: gallori@dbag.unifi.it

Previous studies carried out by our laboratory to understand the characteristics of the interaction between DNA molecules and clay minerals have indicated that the adsorption/binding of nucleic acids by clay crystals could account for the enhancement of the resistance of adsorbed DNA to environmental degradation. This observation could suggest, as well, that the formation of clay-DNA complex have played an important role in the preservation of genetic material in primeval habitats (Franchi *et al.*, 1999).

Here we present the results of further studies on the adsorption/binding process of chromosomal DNA, supercoiled closed circular plasmid DNA and 25S RNA on clay minerals, montmorillonite (M) and kaolinite (K).

Adsorption experiments performed in the presence of different Ca^{2+} concentrations indicated that an increase in the cation concentration produce an increase in the adsorption of the nucleic acid, suggesting that a cation exchange mechanism is involved in the adsorption.

Moreover, Scatchard plot analysis of adsorption isotherms indicated the presence of – at least – two specific types of binding site on clay surface.

To investigate the nature of the nucleic acid interacting sites, we treated M and K with a competitor of the phosphate group of the nucleic acid backbone, as the sodium-exametaphosphate (SMP). Results obtained indicated that SMP reduced significantly the nucleic acid adsorption, confirming the role of the phosphate group in the adsorption/binding process.

Franchi M., Bramanti E., Morassi Bonzi L., Orioli P.L.. Vettori C., Gallori E.: 1999, Origins Life Evol Biosphere, (in press).

P4.11

FORMATION OF FOUR ISOMERS AT ASP-151 RESIDUE OF HUMAN αA-CRYSTALLIN DURING NATURAL AGING

Noriko Fujii[a], Yuko Momose[b], Larry J. Takemoto [c] and Mitsuhiko Akaboshi[a]

a) Research Reactor Institute, Kyoto University, Sennan, Osaka 590-0494, Japan, b)National Institute for Advanced Interdisciplinary Research (NAIR), Tsukuba, Ibaraki 305-8562, Japan, c) Division of Biology, Kansas State University, Manhattan, Kansas, 66506-4901, USA

Proteins have been considered to consist exclusively of L-amino acids in living tissues. However, our previous studies showed that the Asp-151 residue in αA-crystallin inverts to the D-isomer in human, bovine, and horse eye lens during the aging. The high site-specific racemization of the Asp-151 residues in these αA-crystallins suggests that the Asp residues are stereochemically labile in the protein. Especially in human αA-crystallin, the D/L ratio of Asp-151 residue was higher than 1.0. Since racemization is defined as a reversible first order reaction, when D/L ratio reaches 1.0, racemization is in equilibrium. Therefore, a D/L ratio higher than 1.0 is not considered to be due to racemization, but rather results from stereoconfiguration inversion. Our report was the first observation that inversion occurred in the configuration of amino acids in vivo during the natural aging process. On the other hand, we also found that these enantiomers were simultaneously isomerized to form β-Asp (isoaspartate) residue. We report here the change of the ratio of four isomers of Asp-151, normal L-α-Asp, biologically uncommon L-β-Asp, D-α-Asp and D-β-Asp caused from natural aging process.

αA-Crystallins were purified from human lenses of 0, 30, 60- and 80 year ranges. These samples were digested with trypsin and were purified by reverse-phase high pressure liquid chromatography (RP-HPLC). The resulting peptides were characterized by amino acid composition, sequence analysis, and mass spectrometry. We identified Asp-151 containing peptides, that is T18 peptides, and determined the D/L ratios of Asp-151 of the samples in each age. In addition, β- and α-Asp containing peptides can be separated to two peaks on RP-HPLC and the linkages can distinguished by amino acid sequence analysis because β-linkage- amino acid containing peptides are resistant to Edman degradation. Biologically uncommon isomers increased with age and in the αA-crystallin of the human lenses of 80's, D-β-Asp was more than normal L-α-Asp. This modification of the Asp residue likely affects the three-dimensional packing array of the lens protein. We proposed that D-amino acid, which was eliminated in chemical evolutionary process, could be a molecular indicator of aging of the L-amino acid organism.

P4.12

USING mRNA DISPLAY TO EXPLORE PROTEIN SPACE

Anthony D. Keefe and Jack W. Szostak
Harvard Medical School Department of Genetics
Massachusetts General Hospital, Department of Molecular Biology
Boston, MA 02114, USA

The use of *in vitro* evolution for the production of new functional RNA and DNA molecules is now widespread. However, no biochemical mechanism exists for the replication or amplification of proteins and so protein selections cannot be performed in an analogous manner.

Recently Roberts and Szostak (1997) have suggested a mechanism by which protein selections may be performed. In this method a stable covalent bond is formed during *in vitro* translation between a protein and the mRNA that encodes it. Prior to translation, the 3' end of the RNA is ligated to a short DNA oligomer which has puromycin attached to it's 3' end. Puromycin is a translation inhibitor that resembles a charged tRNA and is able to form a stable amide bond with the nascent peptide within the ribosome. When the ribosome encounters the RNA-DNA junction it pauses and the puromycin enters the A-site and forms an amide bond with the C-terminal amino acid of the peptide. Since the puromycin is covalently attached to the mRNA, the mRNA and protein for which it codes are now linked by a stable covalent bond. The RNA-DNA-puromycin-protein construct that results from this approach may be used for *in vitro* selection.

A random sequence library was designed using a weighted iterative computer program (New, M., NASA Ames) to generate a nucleotide distribution for an appropriate amino acid distribution. This was synthesized on a DNA synthesizer using standard phosphoramidite chemistry which resulted in a deletion rate of 1.3% per nucleotide. Deletions result in frameshifts in the expressed protein. Were the 372 nucleotide library to have been synthesized using this DNA directly then only 0.8% would have encoded full length proteins, and only 0.014% would additionally been free of stop codons. In order to separate the molecules with deletions and/or stop codons away from those without either, the pool was initially synthesized in smaller cassettes. Each cassette contained both a FLAG tag at it's 5'-end and a His-tag at it's 3'-end. Translating these sequences and fusing them to the proteins they code for meant that only those which were without frameshifts contained both tags. Purifying these away from the rest upon both anti-FLAG and Ni-NTA columns resulted in a pool of RNA greatly depleted of both stop codons and deletions. The resultant molecules were reverse transcribed and amplified, this DNA was then ligated together to give a library coding for 109 amino acids with a random region of 81 amino acids and a diversity of 4×10^{14}.

P4.12 continued

USING mRNA DISPLAY TO EXPLORE PROTEIN SPACE (cont.)

The selection of RNA molecules free of deletions and stop codons shows that mRNA display can be used to select proteins with desired properties.

This library is currently being used to select for a protein ATP aptamer. ATP was chosen as a target because of it's central role as the energy currency of most biological reactions. A protein ATP aptamer would also be a very suitable starting point for the selection of ATP-powered enzymes.

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Roberts, R. W. and Szostak, J. W.: 1997 *Proc. Natl. Acad. Sci. USA* **94**, 12297-12302.

P4.13

FUTURE OF SEMISYNTHETIC LIFE

O.P.H. Khandelwal, 101, Happy Apartment, 16 E, Sadhna Nagar,
Indore - 452005 (M.P.), INDIA, e-mail : o_khandelwal@hotmail.com

Key Word : Semi Synthetic Life, Synthetic Self Replicating Molecule, Autocatalystic.

If we synthesize life into laboratory the result will be most surprising for a biochemist and evolutionary biologist. The fact is that we can't imagine how complexity in life is involved. "If one can deduce the natural laws that operate to create life on the earth. Why not just assemble the necessary materials and recreat a Life in test-tube?" (Eigen, 1981) If we begin to form biochemical enzymes, proteins, RNAs and DNA we can't constitute life at present. In future, I can't say it will be impossible , but it will be most difficult task. Fred Hoyle and other scientists have said that "even apparently miraculous events become possible such as the spontaneous emergence of single-cell organisim from random coupling of chemicals. Hoyle said such an occurance is about as likely as the assemble of a 747 by tornado whirling through a junkyard." (Johan, 1991, Ruse, 1997).

We can just imagine how we can get semi synthatic life. If we disintegrate a single cell bacteria and again try to put all the biochemicals into another cell-membrane which is empty by means of microsurgical process, we may not get success. But, if we try many-many times with in change order, we may get all assemblage into orderly form to perform all activities of life. If condition remain the same (Kuhan, 1972) So it will be a rare case to synthesize semisynthetic life.

According to Kauffman "the computer simulations demonstrate that a system supplied with a sufficient number of polymers, will undergo a `phase-transition', that causes to become autocatalytic. That is the system will spontaneously begain generating polymers or even greater complexity and catalytic capacity. Kauffman replies to a question about test-tube results : "no one has done this in pot, but I am sure I am right." (Kauffman, 1986, Johan, 1997) After some year of his experiment Julious Rebek jr. got result of synthetic self replicating molecule successfully (Julius, 1994) . But our goal is far away to synthesize semi-synthetic life.

If we are lacking **SOMETHING** we will never get life assemblage from all biochemicals and bioenergetic chemicals. Let us try to know what is this **SOMETHING** by our devoted efferts

P4.13 continued

Eigen, M., **1981**, et.al., Scientific American **April 1981**, p. 78 - 94.

Deamer D.W. **1997**, Microbiology and Molecular Biology Reviews, **June 1997**, p. 239 - 261

de Duve, C, **1995**, *Vital Dust : Life as a Cosmic Imperative*. New York. Basic Books.

de Duve, C, **1991**.Blueprint for a cell : *The nature and origin of life*. Burlington, N.C. Niel Paterson Publisher, Carolina Biological supply Co.

de Duve, C, **1995**, American Scientist, 83 : 428 - 437.

John. Horgan, **1991**, Hoyel and Kauffman in "In the beginning". Scientific American. Feb. 1991. P.116 - 125.

Julius Rebek jr., **1994**, Scientific American. July 1994, P. 48 -55.

Kauffman, S.A., **1986** : J. Theor. Biol. **119** ; 1 - 24.

Kuhan, H., **1972**, Angew. Chem. Internet Edit. Vol - II (1972) No. 9, P. 798 - 820.

Nakamura, H, **1988** : *In report of special research project on evolution of matter.* (Tusuba university)

Ruse M, **1997** : J. Theor. Biol. **187**; 473-482.

P4.14

MOLECULAR PARASITES THAT EVOLVE LONGER GENOMES

Kristin A. Marshall and Andrew D. Ellington
University of Texas at Austin, Austin, TX, USA 78712

Molecular parasites that utilize the replication machinery of cells or of in vitro amplification reactions have previously been characterized. By and large, these parasites have been smaller than the viruses or amplicons that gave rise to them. This is likely because shorter genomes can be replicated more quickly. In contrast, we have identified and characterized parasites of an isothermal amplification reaction that are longer than their parental molecules yet replicate much more efficiently. These results raise interesting questions regarding whether the optimal size of replicators reflects a trade-off between the information encoded in a parasite versus the information encoded in the machinery replicating that parasite.

We initially set out to develop an automated in vitro selection protocol. To this end, we decided to use a fast, continuous amplification method (3SR). 3SR mimics retroviral amplification of RNA by use of a cDNA intermediate. This method has been used by numerous researchers for amplification of target RNA sequences *in vitro*. However, when this method was adapted to selections that started from completely random sequence populations, molecular parasites with genomes longer than those of the starting RNA species quickly evolved. The parasite was originally present as a single band, but in later experiments a second, larger band appeared. The length of the parasites appears to be functional rather than incidental, as they replicate much faster than their forebears. Throughout its genesis and evolution, the artefact proved to be a true parasite of the isothermal amplification reaction. It required both primers, reverse transcriptase, and T7 RNA polymerase for its replication. The smaller, Class I parasite seems to represent ca. 3 ½ repeats of a 23 residue core sequence. The larger, Class II parasite appeared to be derived from two different members of the original random sequence pool which had joined head-to-tail and in the process deleted a portion of their constant regions.

While the mechanism of replication of theses parasites is currently unknown, it is likely the case that individual sequences were too small to be optimally replicable within an isothermal amplification reaction, and that these sequences augmented their information content in order to increase their replicability.

P4.15

"EARLY" EUKARYOTES? - MOLECULAR PHYLOGENETICS OF THE VACUOLAR ATPASE AND THE SEARCH FOR EUKARYOTIC ORIGINS.

Lorraine Olendzenski and J. Peter Gogarten

Dept. of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA

Accurate eukaryotic phylogenies are requisite to our understanding of eukaryotic evolution in that they can provide a basis for understanding the evolution of phenotypic innovation and can give clues to the biology of the earliest eukaryotic cells. Phylogenetic analyses based on small subunit (SSU) ribosomal RNA sequences consistently group the amitochondriate diplomonads, trichomonads and microsporidia at the base of the eukaryotic lineage. Recently, the phylogenetic position of microsporidia has been called into question. Although SSU rRNA (Liepe et al., 1993) and elongation factor analyses (Kamaishi et al., 1996) place these microorganisms as one of the most basal eukaryotic lineages, phylogenetic analyses of tubulins (Edlind et al., 1996, Keeling and Doolittle, 1996) and RNA polymerase sequences (Hirt et al., 1999) suggest that microsporidia are most closely related to fungi. To help resolve the phylogenetic position of the microsporidia, and to discern to what extent their position at the base of ribosomal RNA phylogenies may be due to long branch artifacts, we have sequenced the catalytic subunit of the V-type ATPases from the microsporidian *Nosema locustae* and the trichomonad *Trichomonas vaginalis*. Preliminary analyses of available eukaryotic A subunits consistently group the microsporidial sequence with the fungi and place *Giardia* at the base of the eukaryotes.

To test the hypothesis that the basal position of the microsporidia in SSU rRNA trees may be a long branch artifact, we investigated how likelihood values change for tree topologies in which a branch containing microsporidia is moved into different positions in the tree, from the base to the 'crown'. Using the SSU rRNA dataset, we started with an accepted topology of the eukaryotes, moved a branch containing the microsporidia to various places within it, and calculated the likelihood of each tree topology using PUZZLE 4.0. Although SSU rRNA trees which place microsporidia at the base of eukaryotes have the highest likelihood, there is a slight increase in likelihood for trees which group microsporidia with the animals or fungi, suggesting that there is a signal within the SSU rRNA dataset that supports the grouping of microsporidia with the fungi.

This dual affinity of the microsporidia for two locations within SSU rRNA phylogenies argues against interpreting the conflicting phylogenies obtained with V-ATPases and other markers as a result of horizontal gene

P4.15 continued

EARLY EUKARYOTES? - MOLECULAR PHYLOGENETICS OF THE VACUOLAR ATPASE (con't.)

transfer (Gogarten et al., 1996), (i.e., the microsporidia represent a deep branching lineage that might have picked up a V-ATPase from a fungal host they were parasitizing). Rather it appears that the deep position of microsporidia in SSU rRNA trees reflects a long branch attraction. The magnitude of this apparent artifact necessitates a rethinking and critical evaluation of other deep relationships.

Edlind, T.D., Li, J., Visversvara, G.S., Vodkin, M.H., McLaughlin, G.L. and Katiyar, S.K.: 1996, Mol. Phylogenet. Evol. **5**, 359-367.

Gogarten J.P., Hilario E., and Olendzenski, L.: 1996 In: *Evolution of Microbial Life* , Society for General Microbiology **54**, 268-292.

Hirt, R.P., Logsdon, J.M., Healy, B., Dorey, M.W., Doolittle W.F. and Embley, M.T.: 1999, Proc. Nat. Acad. Sci. USA **96**, 580-585.

Kamaishi, T., Hashimoto, T., Nakamura, Y., Nakamura, F., Murata, S., Okada, N., Okamoto, K., Shimizu, M., and Hasegawa, M.: 1996, J. Mol. Evol. **42**, 257-263.

Keeling, P.J. and Doolittle, W.F.: 1996, Mol, Biol. Evol. **13**, 1297-1305.

Leipe D. D., Gunderson, J. H. Nerad, T. A. and Sogin M. L.: 1993, Mol. Biochem. Parasitol. **59**, 41-8.

P4.16

ORIGIN OF BACTERIAL OUTER MEMBRANE

Martino Rizzotti
Department of Biology, University of Padova, Italy, rizzotti@bio.unipd.it

The so-called Gram-negative Eubacteria (G-) are the sole cells endowed with an outer membrane which surrounds the plasma one. The constant functional property of the outer membrane seems to be its permeability to low-molecular-weight hydrophilic molecules, including monoatomic ions (H^+, Na^+, Cl^-, etc.). This is due to the presence of *porins*, which are thus presumed to be encoded by all genomes of G-. Moreover, the growth of the outer membrane requires dedicated morphogenetic factors, which thus make G- more complex, other things being equal, than other Prokaryotes.

The origin of the outer membrane has been supposed to have occurred in the first cell as the result of deformation of a lipid vesicle (Blobel, 1980). At the very beginning, according to this hypothesis, it was a standard membrane, but it very rapidly acquired its typical functional property. Later on, it was lost one or more times, allowing G+ Eubacteria, Archaeobacteria and Eukaryotes to arise. In any case, its morphogenetic requirements render it unlikely to have appeared and been handed down so early. This hypothesis also requires that the primordial hydrosphere already contained, in addition to membranogenic lipids, polynucleotides, enzymes, and translation machinery, all of which are also very implausible and qualify this hypothesis as the most demanding one for the environment in which the first cell appeared.

Alternatively, the origin of the outer membrane may have occurred in an advanced, albeit ancient, bacterium when morphogenetic regulation was already of a modern kind (Rizzotti, 1999). According to this hypothesis, the first Prokaryotes had no outer membrane. It gradually appeared starting from selected folds of the plasma membrane, probably because they reduced diffusion of digestive enzymes in the environment. The outer membrane was never lost, except perhaps in stabilized endosymbioses (mitochondria and plastids). This hypothesis is in accordance with the stepwise complexification of early cells.

Blobel, G.: 1980, Proc. Natl. Acad. Sci. USA 77, 1496.
Rizzotti, M.: 1999, *Early evolution*, Birkhäuser, Basel.

P4.18

IDENTIFICATION AND CHARACTERIZATION OF GROUP I INTRONS IN DEEP SUBSURFACE BACTERIA

Alexey A. Vepritskiy, Jennifer C. Reineke, Marc E. Frischer, Sandra A. Nierzwicki-Bauer. Department of Biology, Rensselaer Polytechnic Institute, MRC 306, Troy, NY 12180

A controversial issue regarding the origins of life, which remains unsolved, is whether group I self-splicing introns are ancient features of gene structure or whether they arose more recently. If group I introns are indeed molecular fossils then it is predicted that they should be present in all DNA-RNA-protein life which arose from an RNA universal ancestor.

Although introns in eukaryotes, archaebacteria, and viruses are well represented, our understanding of their distribution in eubacteria is limited. Determining the phylogenetic distribution of introns in eubacteria is a valuable tool to assess their evolutionary history. Identification and study of introns from the widest possible diversity of cells may give new insights into the temporal sequence and evolutionary relationships of life on Earth. In this regard bacteria derived from deep subsurface environments, which may have been isolated from modern populations for hundreds of millions of years, could provide a unique source of intron sequences mostly lost from surface populations.

We are searching for group I introns in different deep subsurface eubacterial isolates, identified in our laboratory as pseudomonads. DNAs from fifty such isolates were screened with an oligonucleotide probe targeted to the conserved Q-region for all known group I self-splicing introns. Thirteen isolates, that displayed positive hybridization signal with the probe, when analyzed by RFLP revealed seven unique banding patterns, which strongly correlated with the depth from which these bacteria had been obtained. This may indicate that there has not been significant transport of these bacteria between different sediment types. To date, all published eubacterial group I introns are found to reside within tRNA genes. Thus, we are currently exploring the possible presence of group I introns in our subsurface isolates within a variety of tRNAs, using PCR amplification and appropriate primer sets. Positive amplification products are being cloned and sequenced.

P4.19

COMPARATIVE ANALYSIS OF THE DEINOCOCCUS GENOME INDICATES INFREQUENT HORIZONTAL TRANSFER BETWEEN THE THREE DOMAINS OF LIFE

Olga Zhaxybayeva, Lorraine Olendzenski, Peter Gogarten
Dept. of Molecular and Cell Biology, Univ. of Connecticut, Storrs, CT

Lei Liu
W. M. Keck Center, University of Illinois, Urbana, IL

Members of the deep branching bacterial lineage *Deinococcaceae* (e.g. *Thermus* and *Deinococcus*) are classified as Bacteria based on cell wall, lipid composition, and ribosomal RNA sequence (Hensel et al., 1986). However, *Thermus thermophilus* does not have an F-ATPase like other bacteria, but a complete A/V-ATPase (Yokoyama et al., 1990; Tsutsumi et al., 1991). A-ATPases are usually found only in archaea while V-ATPases are usually found only in eukaryotes. The typical ATPase of bacteria is the F-ATPase, although A/V type ATPases have been found in spirochetes, *Enterococcus*, and *Chlamydia*. The majority of *Thermus* and *Deinococcus* species examined thus far contain only an A/V-ATPase, although a recent report indicates that *Thermus scotoductus* contains an F-ATPase (Radax et al., 1998). The vacular/acrchaeal type ATPase genes are not the only evidence of non-bacterial type genes in the *Deinococcaceae*. *Thermus flavus* was shown to have a malate dehydrogenase that in phylogenetic analyses groups with the eukaryotic homologues (Iwabe et al., 1989). Genetic screening of a *Thermus ruber* genomic library with randomly primed probes made from *Thermoplasma acidophilus* genomic DNA identified a number of clones which hybridized to the archaeal probes. One of these encoded a prolyl-tRNA synthetase, which was shown to be more closely related to archael and eukaryotic type prolyl tRNA synthetases than to the majority of bacterial prolyl tRNA synthetases (Ryan Murphey, personal communication). The most plausible explanation for the presence of non-bacterial genes in members of the *Deinococcaceae* is horizontal gene transfer from the archaeal lineage to this bacterial lineage (Olendzenski et al., 1998).

P4.19 continued

COMPARATIVE ANALYSIS OF THE DEINOCOCCUS GENOME INDICATES INFREQUENT HORIZONTAL TRANSFER (con't.)

This hypothesis led us to use a bioinformatics approach to search for other candidate genes in the *Deinococcus radiodurans* genome that might have been horizontally transferred from the archaeal domain. The genome of *D. radiodurans* has been completely sequenced, partially assembled, and is available in preliminary form through the TIGR web-page (http://www.TIGR.org). Putative open reading frames (ORFs) from the *Deinococcus* genome were searched against representative completed genomes from each of the three domains of life. Those ORFs that showed highest matches against archaeal and eukaryotic genes were collected and ranked. Among the top ranked hits were the A/V-ATPase catalytic and non-catalytic subunits, and the prolyl-tRNA synthetase. Additionally, the high scores obtained in searches against the *Saccharomyces*, *Methanococcus*, and *Escherichia* genomes were plotted in a 3-D graph. Putatively frequently transferred genes are those which reside on or close to the line which is equally distant from all three axes.

This approach can be applied to different genomes and might provide a means to classify genes as to the frequency of horizontal transfer among the three domains of life.

Hensel, R., Demharter, W., Kandler, O., Kroppenstedt, M., Stackebrandt, E.:1986, Internatl. J. Syst. Bact. **36**, 444-453

Iwabe, N., Kuna, K.I., Hasegawa, M., Osawa, S., Miyata, T.: 1989, Proc. Natl. Acad. Sci., USA **86**, 9355-9359

Olendzenski, L., Hilario, E., Gogarten, J.P.: 1998, In: *Horizontal Gene Transfer*, Chapman and Hall, London. pp 349-362

Radax, C., Sigurdsson, O., Hreggvidsson, G.O., Aichinger, N., Gruber, C., Kristjansson, J.K., Stan-Lotter, H.: 1998, Syst. Appl. Microbiol. **21**, 12-22

Tsutsumi, S., Denda, K., Yokoyama, K., Oshima, T., Date, T., Yoshida, M.: 1991, Biochim. Biophys. Acta, **1098**, 13-20

Yokoyama, K., Oshima, T., Yoshida, M.: 1990, J. Biol. Chem., **265**, 21946-21950

P4.20

WHOLE GENOME-BASED PHYLOGENETIC ANALYSIS OF MICROORGANISMS

Sorel T. Fitz-Gibbon* and Christopher H. House#
* Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA, 90095-1489
# Department of Earth and Space Sciences and the IGPP Center for Astrobiology, University of California, Los Angeles, CA, 90095-1567
The authors contributed equally to the work

Using the presence and absence of protein encoding genes as phylogenetic characters, we have constructed a "tree of life" using thirteen complete microbial genomes. This analysis is based on the entire genome, not just the few universally conserved genes, and the tree produced is nonetheless very similar to the small subunit rRNA tree[1]. Our result is in contrast to notions that a robust phylogenetic reconstruction of complete microorganisms is impossible due to their genomes being composed of an incomprehensible amalgam of genes with complicated histories.

Using the data from all published genomes larger than 1.5 Mb and the 2.2 Mb unpublished genome of *Pyrobaculum aerophilum*, we grouped proteins based on pairwise sequence similarity. The presence or absence of each group was then scored for each genome to construct the data matrix for phylogenetic analysis.

Parsimony and distance analyses were performed for a range of Z-score cutoffs. The consensus topology was well supported by the data, as indicated by high bootstrap values (mostly 100), consistency indices, decay indices, and the consistency across differing Z-score cutoffs. Some of the bacterial domain is unresolved, in accordance with the poor resolution for these groups in small subunit rRNA analyses, suggesting that these organisms underwent a rapid radiation from their last common ancestor.

In contrast, each branch of the archaeal domain is clearly resolved. Surprisingly, the two methanogens included in this study form a monophyletic group to the exclusion of Archaeoglobus, whereas in small subunit rRNA trees the methanogens are paraphyletic, with *Methanobacterium* as the sister taxon to *Archaeoglobus*. The support for the novel pairing we observed is dominated by the presence of 85 gene groups (at a Z-score cutoff of 200) unique to the methanogen genomes, which include 68 protein groups of unknown function, two operons involved in methanogenesis (mcr, mtr), and a seryl-tRNA synthetase group.

Woese, C. R., Kandler, O. & Wheelis, M. L. :1990, Proc Natl Acad Sci USA **87**, 4576-9.

P4.22

COEVOLUTION OF PEPTIDES AND NUCLEIC ACIDS
Interhelical Organization as possible Origin of the Triplet Code and Homochirality

Bettina Heinz

California State University San Marcos /Chemistry, CSUSM, San Marcos CA, USA 92069

Early life could have been induced by the interplay of large biomolecules. Coevolution of peptides by way of interlocking helices is considered a possible factor for the origin of the triplet code as well as the homochirality of the amino acids.

It takes ten bases and 3.6 amino acids to make one full turn in their respective helical configurations. This ratio of one amino acid to 2.8 bases would be pushed to the 1:3 triplet code relationship by bulging effects of the concentric helical hybrid. The correct code words would not only be established by hydrogen bonding (the central base being the critical and consistent amino acid binding element with variable code words) but more prominently by molecular orbital energy minimization and symmetry conservation criteria. This means that a maximum of constructive LUMO and HOMO overlaps with minimum of nodal planes would be achieved.

The electron flux along the right-handed alpha-helical strand of the polypeptide produces an oscillatory current that inherently could favor the L-form of the amino acids. D-amino acids would be reserved for invasive strategies in developed life forms. Circular polarized radiation drives electrons in a helical path by which oscillating electrical and magnetic fields parallel to the helix axis are produced. Rotatory behavior of the helical molecules can thus result in optical activity for the electron transitions in the peptide chain itself or in the amino acid side groups. The asymmetric environment can also produce distorted fields in the neighboring heterocyclic bases. Circulatory circuits of the peptide filaments thus depend on the homochirality of the amino acids.

Flexible hydrogen bonds allow the center peptide coil to move along the axis of the outer single or within the double coil of the nucleic acid leading to a molecular Tesla coil system (primary and secondary coil). This implies that molecular filaments are capable of producing signals and transmitting electromagnetic information into the prebiotic environment, resulting in further self-assembly and morphogenesis.

In laboratory experiments tightly coiled peptides consisting of 6 to 10 residues, sometimes connected to fluorescent chromophores, are exposed to single strand, but complementary oligonucleotides (18-30 bases) and interhelicalization monitored by spectroscopic methods. In other studies specifically synthesized cyclic trinucleotides that according to molecular modeling principles produce cage molecules with crown ether properties are subjected to single amino acids in the presence of metallic divalent ions to see if any natural affinity to these individual amino acids by way of encapsulation occurs.

P4.23
EVOLUTION OF PROTEIN SYNTHESIS

W. J. M. F. Collis
Strada Sottopiazzo, 18, 14056 Boglietto (AT), ITALY (mr.collis@physics.org)

The genetic code is almost universal and this uniformity makes it difficult to speculate upon its origin and subsequent evolution. By analysing the amino-acid composition of ancient aminoacyl-tRNA synthetase enzymes (AARS) as extrapolated from known modern sequences, this paper illustrates a method of estimating the temporal order in which specific amino-acids were first incorporated into proteins. This gives new insights into the evolution of the early genetic code and archaic protein synthesis.

The results show that the two AARS classes did not co-evolve as has been speculated, but that the class I developed using an archaic subset of class II AARS. The data suggest that the first AARS enzymes were bootstrapped from even earlier protein synthesis machinery, possibly of rybozymal origin.

i5.1

THE EVOLUTION OF EARLY SOLAR SYSTEM ANALOGS

David W. Koerner
Department of Physics and Astronomy, University of Pennsylvania, 4N14 David Rittenhouse Laboratory, 209 South 33rd Street, Philadelphia, PA 19104-6396

Until recently, efforts to understand the chemical origin of life were impeded by ignorance of the true conditions in the early solar system and on the young earth. Organics like those discovered in the interstellar medium may have played a strong role in terrestrial biogenesis, for example, but this cannot be confidently assumed without clear evidence that they survived the planet-forming process. These and similar issues are now addressed by high-resolution imaging of the protoplanetary environment around young stars which serve as analogs of the early solar system (Koerner 1997). Millimeter arrays provide maps of the dust and molecular gas for young stellar objects as they evolve from the earliest phase of protostellar collapse to the point at which viscous accretion subsides (Koerner et al. 1993; 1995; Velusamy et al. 1995). Optical and near-infrared images from Hubble Space Telescope yield a detailed look at the morphology of the disk surface illuminated by light from the central star or by back-illumination from bright nebulae (Burrows et al. 1996; McCaughrean and O'Dell 1996; Padgett et al. 1999). Thermal infrared imaging of circumstellar dust traces the subsequent evolution of planetesimals after the nebular gas has largely dispersed (Lagage and Pantin 1994; Koerner et al. 1998). These observations comprise an ensemble of measurements from which the evolving properties of a protoplanetary system can be reconstructed. Observed disk sizes indicate that much of the accreting material enters a protoplanetary disk at large radial distances (> 50 AU) with sufficiently low velocity to ensure preservation of organic molecules. The properties of gas-free disks support the notion that this gas condenses as an outer "Kuiper Belt" zone of cometesimals from which organic-rich icy bodies may be delivered to a terrestrial planet region.

i5.1 continued

Organic detections in disks include CN, HCN, and HNC in the gas phase (Dutrey et al. 1997), and Polycyclic Aromatic Hydocarbons in dust grains (Ressler and Barsony 1999). Future observations will greatly expand our knowledge of the precise radial distribution and abundance of dust and gas-phase molecules and, as a result, narrow down the range of properties expected for the earliest atmospheres and oceans of terrestrial planets.

Burrows, C. J., Stapelfeldt, K.R., Watson, A.M., et al.: 1996, Astrophys. Journ. 473, 437.

Dutrey, A., Guilloteau, S., and Guilin, M.: 1997, Astron. & Astrophys. 317, L55.

Koerner, D. W., Sargent, A. I., and Beckwith, S. V. W.: 1993, Icarus 14, 2.

Koerner, D. W., and Sargent, A. I.: 1995, Astron. Journ. 109, 2138.

Koerner, D. W., Ressler, M. E., Werner, M. W., and Backman, D. E.: 1998, Astrophys. Journ. 503, L83.

Koerner, D. W.: 1997, Orig. Life and Evol. Bio. 17, 157.

Lagage, P. O. and Pantin E.: 1994, Nature 369, 628.

McCaughrean, M. J., and O'Dell, C. R.: 1996, Astron. Jour. 111, 1977.

Padgett, D. L., Brandner, W., Stapelfeldt, K. R., et al.: 1999, Astron. Journ., in Press.

Ressler, M. E., and Barsony, M.: 1999, Astrophys. Journ., Submitted.

Velusamy, T., Kuiper, T. B. H., and Langer, W. D.: 1995, Astrophys. Journ. 451, L75.

i5.2

SURVIVAL AND BIOLOGICAL EVOLUTION OF LIFE BEYOND ITS PLANET OF ORIGIN

Christopher P. McKay, NASA Ames, Moffett Field CA, 94035, USA

One of the key questions in Astrobiology is the ability of life to expand beyond its planet of origin. Understanding the mechanisms by which life can spread from planet to planet and even from star system to star system may be relevant to the origin of life on Earth and Mars (Davis and McKay, 1996) and is certainly relevant to the future role in the cosmos of life from Earth. Recent work on meteorites show that there are natural processes that spread life from planet to planet (Bogard and Johnson, 1983; Melosh, 1988; McSween, 1994). Meteorites on Earth from Mars clearly demonstrate the efficacy of interplanetary delivery processes. Models suggest that some fraction of the debris ejected into space by an impact should transit from Mars to Earth in timescales of a few years even though the majority of the material has transit times over ten million years (Gladman *et al.*, 1996). Even for these longer transit times it is possible that microorganism could remain viable in a dehydrated dormant state. Long-term survival on Earth has been demonstrated in amber for 25 Myr (Cano and Borucki, 1995) and in permafrost for over 3 Myr (Gilichinsky *et al.*, 1992) with even longer survivals speculated in salt (200 Myr). Dust grains ejected by impacts could be carried out of the solar system and provide a mechanism for life spreading from star to star. In additional to natural processes, the spread of life beyond the planet of origin could be the result of activity by an intelligent species. In our own solar system the first opportunity for this could be the introduction of life on Mars; microbes, plants, and ultimately animals (McKay *et al.*, 1991).

Bogard, D. D. and Johnson, P.: 1983, *Science* **221**, 651-654.

Cano, R. J. and M. K. Borucki.: 1995, *Science* **268**, 1060-1064.

Davis, W. L. and McKay, C. P.: 1996, *Origins Life Evol. Biosph.* **26**, 61-73.

Gladman, B. J., Burns, J. A, Duncan, M., Lee, R., and Levison, H. F.: 1996, *Science* **271**, 1387-1392.

Gilichinsky, D. A., Vorobyova, E. A., Erokhina, L. G., Fyordorov-Dayvdov, D. G., and Chaikovskaya, N. R.: 1992, *Adv. Space Res.* **12**, 4:255-263.

McKay, C. P., Toon, O. B., and Kasting, J. F.: 1991, *Nature* **352**, 489-496.

McSween, H. Y.: 1994, *Meteoritics* **29**, 757-779.

Melosh, H. J.: 1988, *Nature* **332**, 687-688.

i5.3

SEARCHING FOR EXTRATERRESTRIAL LIFE: LESSONS FROM THE EARTH

Kenneth H. Nealson
Senior Research Scientist, Jet Propulsion Laboratory, 4800 Oak Grove Drive, Pasadena, CA 91109, USA

The search for extraterrestrial life has become a popular item of discussion in the past few years, and with the upcoming sample return missions from Mars, the possibility that pristine extraterrestrial samples could be examined has increased the excitement. The reasons for the optimism in this search are based on a variety of findings in planetary research, ranging from the discovery of extrasolar planets around far away stars, to the evidence for an ice covered moon of Europa (perhaps containing a sub-ice ocean), to direct evidence for an active hydrological cycle on Mars. Some optimism can also be derived from studies of life on Earth, new insights into the time of the earliest life on Earth, into the toughness and tenacity of Earthly life, and into the metabolic diversity exhibited by Earthly life. Taken together, these lessons from the Earth suggest that our definition of habitability may need to be expanded, and that a combination of knowledge of the planetary body being studied, along with the types of metabolism that might be expected to reside there form should be employed in designing a strategy for the search for life.

c5.4

FROM INTERSTELLAR DUST VIA COMETS TO LIFE ?

Pascale Ehrenfreund (1), Adwin Boogert (2), Achim Enzian (3),
Perry Gerakines (4) and Bernard Foing (5)
(1) Leiden Observatory, P O Box 9513, 2300 RA Leiden, Holland
(2) Caltech, Pasadena (3) JPL, Pasadena (4) NASA Goddard, Greenbelt
(5) ESA Space Science Department

More than 120 interstellar and circumstellar molecules are currently identified in the gas phase along with a small fraction of interstellar dust composed of a mixture of materials from various cosmic sources (Spaans & Ehrenfreund 1999). Recent ground-based observations and satellite data from the Infrared Space Observatory ISO have provided revolutionary results concerning the nature of cosmic dust particles. Interstellar grains act as an important catalyst in the interstellar medium. Processes such as ultraviolet irradiation, cosmic ray bombardment and temperature variations determine the grain mantle growth and chemical evolution.

The incorporation of interstellar matter in meteorites and comets in the pre-solar nebula provides the basis for the "cosmic dust connection" (Ehrenfreund 1999). A comparison of interstellar and cometary dust using recent ISO data and ground-based measurements has revealed important similarities but also indicated that comets contain beside pristine interstellar material, admixtures of processed material (Ehrenfreund et al. 1997, Ehrenfreund 2000). The investigation of molecules in interstellar clouds and comets is essential to reveal the link between dust in the interstellar medium and in the Solar System and provides important clues on the prebiotic chemical evolution on Earth.

In space most of the chemical evolution toward complex molecules takes place in the solid phase, particularly accessible to laboratory simulations. We present laboratory data relevant to ultraviolet irradiation, cosmic ray bombardment and thermal processing of dust. This allows to reconstruct the conditions in the protostellar environment and to monitor the evolution of simple carbon bearing species to complex molecules and aromatic networks. New results indicate in fact a lack of radiation processing in dense clouds, which may strongly decrease the yield of organics formed in those environments (Ehrenfreund et al. 1998). We present studies on the outgassing properties of bright comets (such as Hale-Bopp and Hyakutake) which are compared to recent interstellar dust model predictions. We critically discuss whether interstellar molecules brought by comets may act as precursors for biogenic molecules.

Ehrenfreund, P. et al. 1997, Icarus **130**, 1.
Ehrenfreund, P. et al. : 1998, Astron.Astrophys. **339**, L17.
Ehrenfreund, P.: 1999, Science **283**, 1123.
Ehrenfreund P.: 2000, Annual Review of Astron.Astrophys., in preparation
Spaans, M. and Ehrenfreund, P.,: 1999, in: *Laboratory Astrophysics and Space Research*, eds. Ehrenfreund P. et al., Kluwer Academic Publishers, Boston, 1.

c5.5

PLANETARY INTERCHANGE OF BIOACTIVE MATERIAL: RADIATION AND SAMPLE BIASING EFFECTS

Benton C. Clark
Lockheed Martin Astronautics, POB 179, Denver, CO, USA 80201

It is now well-accepted that both lunar and martian materials are represented in the meteorite collections (a dozen or more of each). Early suggestions that viable biological material might survive natural transport (Horneck et al., 1985; Clark, 1985; Melosh, 1988) have not yet been thoroughly examined. No martian meteorite has provided any direct evidence of possible extant biological activity, although one (ALH 84001, Mckay et al., 1996) has unusual characteristics that may indicate ancient metabolic activity. The concept of Planetary Interchange of Bioactive Material (PIBM) has been used to infer that the potential danger to Earth from martian materials is nil, an inference with, however, many pitfalls.

Successful transfer of viable organisms is difficult. In this paper, two particularly important aspects are examined. The space radiation insult includes: ultraviolet, solar particle events (SPE), and galactic cosmic rays (GCR). Solar UV produces lethal effects in hours to days, but only to shallow depths (um-mm) in geological materials. SPE and GCR consist of energetic charged particles (ions), with the latter penetrating deeper but producing lower doses in the first 10's of cm of depth. Transport times (Gladman et al., 1996) can be long compared to space radiation survival times, even at meter depths, according to depth-dose curves constructed for typical geologic materials in free space.

Planetary surfaces consist of igneous rocks and bedrock, of regolith fine particles, of sedimentary deposits, and other oases and specialized eco-niches (protected soils, rock weathering rinds, hydrothermal zones, fumeroles, deep aquifers, ices, etc.). Based upon materials properties, estimated rates of impact of martian materials on Earth and efficiencies for interplanetary transfer (op. cit.), the survival biases of sampling from typical surfaces and specialized locales is evaluated. These analyses provide quantitatively-based constraints on the extent to which PIBM may have played a significant role in transporting viable organisms between Venus-Earth-Mars, both in the early solar system and the current epoch.

Clark, B.: 1985, Orig. Life **16**, 410. Horneck, G. and H. Buecker, ibid., 414. Gladman, B. et al: 1996, Science **271**, 1387. McKay, D. S. et al: ibid., **273**, 924. Melosh, H. J.: 1988, Nature **332**, 687.

c5.6

IDENTIFICATION OF CANDIDATE EXTRATERRESTRIAL ORGANIC BIOMARKERS

G. D. McDonald, A. I. Tsapin, M. C. Storrie-Lombardi, and K. H. Nealson, Jet Propulsion Laboratory, MS 183-301, 4800 Oak Grove Dr., Pasadena, CA 91109

One of the problems in the analysis of extraterrestrial samples for organic signatures of past or present life is the choice of target compounds. Judicious choice of target biomarkers is particularly important when sample size is minimal, since detection limits for various analytical techniques will dictate that assay priorities be established. Variations of specific biomarker compounds among species on Earth may be a consequence of historical evolutionary pathways, and extrapolation of these patterns to putative extraterrestrial biota may not be valid. An extraterrestrial biology, for example, may or may not use DNA or RNA as its genetic material, and it may or may not use amino acid-based protein catalysts. Terrestrial biochemistry cannot be used as a detailed model for possible extraterrestrial life.

There are certain fundamental chemical functions, however, which must be carried out by any system that meets the general definition of a living organism. Some form of compartmentalization is necessary to maintain spatial proximity of components and to allow for generation of chemical potential gradients from which energy can be utilized. Oxidation and reduction reactions, facilitated by electron carriers, must be carried out for energy production and carbon and nitrogen fixation. In environments with low water potential, osmotic balance between cell interiors and the external milleu must be maintained by osmolytes. Some form of cell-cell recognition using quorum sensing compounds may be necessary to maintain optimal cell density and avoid local depletion of resources.

Analyses for compounds such as fatty acids, quinones, amino acids, betaines, and lactones in various terrestrial environments are helping to focus the search for extraterrestrial organic biomarkers. This work is concentrating on extreme environments such as alkaline lakes, permafrost, and deserts, and is being carried out in conjunction with microbiology and molecular biology studies that are assessing the biodiversity of these environments

c5.7

SHEDDING SOME LIGHT ON THE POSSIBILITY OF EUROPAN LIFE

John D. Rummel, NASA Headquarters, Washington, DC 20546; and
Cindy L. Van Dover, The College of William and Mary, 238 Millington Hall, Williamsburg, VA 23187

Recent results from the Galileo Europa Mission (Carr *et al.* 1998) and our expanding knowledge of Earth's deep sea and subsurface biosphere suggest that Jupiter's moon Europa may be possessed of a deep, liquid-water ocean capable of supporting life forms similar to those known on Earth. In 2003, the US is planning to launch an orbiter mission to Europa with the central objective of determining the presence or absence of an Europan ocean. If an ocean is found, the orbiter may also be able to characterize its extent and its relationship to Europa's icy exterior.

Should liquid water exist in quantity beneath the surface of Europa, then the questions of whether there has been an independent origin of life on Europa and whether there is an extant Europan biology are of great importance, not only to exobiologists but to those planning subsequent missions to this intriguing body. In assessing the potential existence of life on Europa there must be a concerted effort to understand the abiotic energy sources and material cycling systems that may be available. Such an understanding will be essential to a projection of the various chemical and physical measurements that could be made to reveal the existence of biological processes in a Europan ocean.

Lessons from the Oceans of Earth. History records that biologists were greatly surprised by both the extent and nature of communities discovered in 1977 at hydrothermal vents (and later at chemical seeps) in the deep sea. A qualitative shift took place in the understanding of life on Earth with the discovery of these communities—a shift that has important implications for the potential for life on (or in) Europa. Yet our understanding of life in the deep sea on Earth is still very much in its youth. For example, no hydrothermal vent communities have yet been studied in either the Indian or Arctic Oceans, and the global biogeographic continuum of hydrothermal vent life remains to be drawn. In particular, the location and study of Arctic Ocean hydrothermal vents, some of which should exist under perennial sea ice, is an important precursor activity to any prospecting for life that may be encountered under the much more challenging conditions on Europa.

On Earth, the quality and quantity of the various sources of energy available to biological systems at deep sea hydrothermal vents continue to be measured and assessed. Investigators have recently quantified the nature of the light available (cf., Van Dover *et al.* 1994) at black smokers and flange pools along ridge systems in the Pacific, and like chemical and thermal

energy these light sources appear to be available for biological exploitation. Since it is possible that one consequence of the tidal heating of Europa would be the formation of tectonic ridges and associated hydrothermal vent systems, including high-temperature vents similar to "black smokers" on Earth, photosynthesis could join chemosynthesis as an energy source available to biological systems in an Europan ocean.

What Can We Do, and When Can We Do It? It is clear that any specific strategy for the exobiological exploration of Europa will have to await the confirmation of an ocean under the surface of that body, as well as data on its chemical composition. But progress can be made in the development of instrumentation important in studying life at deep sea vent systems on Earth (e.g., a special purpose *in situ* spectrofluorometer to characterize energy metabolism of vent microbes) and its eventual miniaturization to meet the mass and power limitations needed for its operation on a space mission. In developing mission hardware for an eventual lander mission and the possible exploration of the Europan ocean, many lessons can be learned through air-dropped tests of a lander, ice-penetration system, and compatible autonomous underwater vehicles capable of the localization and characterization of a hydrothermal vent systems along the Arctic ridge system, under the permanent sea ice on Earth.

Carr, M. H., M. J. S. Belton, C. R. Chapman, M. E. Davies, P. Geissler, R. Greenberg, A. S. McEwen, B. R. Tufts, R. Greeley, R. Sullivan, J. W. Head, R. T. Pappalardo, K. P. Klaasen, T. V. Johnson, J. Kaufman, D. Senske, J. Moore, G. Neukum, G. Schubert, J. A. Burns, P. Thomas and J. Veverka: 1998, Nature **391**, 363.

Van Dover, C. L., J. R. Cann, C. Cavanaugh, S. Chamberlain, J. R. Delaney, D. Janecky, J. Imhoff, J. A. Tyson, and the LITE Workshop Participants: 1994, Eos **75**, 44.

P5.1

PYROLYSIS OF BIOMOLECULES DELIVERED TO THE EARTH BY SPACE BODIES

Vladimir A. Basiuk and Janna Douda
Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México, Circuito Exterior C.U., 04510 México, D.F., MEXICO

The idea of extraterrestrial delivery of organic matter, including biologically important compounds such as amino acids and nucleic acid bases, to the early Earth is now generally recognized. It seems obvious that ubiquitous organic matter found in the interstellar medium, comets and meteorites can be brought to the planetary surface. At the same time, taking into account the fact that space bodies passing through the atmosphere and impacting Earth's surface can be exposed to a significant heating up to total decomposition of organic species, complicates the problem. Especially it is true in the case of amino acids and nucleic acid bases, vital for the life but considered thermally unstable compounds.

Nevertheless, there are indisputable proofs that these biomolecules still can reach Earth's surface: carbonaceous chondrites, where a variety of amino acids, as well as some purines and pyrimidines have been detected. In some cases the content of amino acids reaches very considerable levels, of the order of 1-10 $\mu g\ g^{-1}$, demonstrating that the biomolecules can be efficiently protected of the heat inside such space bodies. On the other hand, contribution (at least present day) of meteoritic organics is estimated to be a few kilograms per year only.

Other kinds of space bodies to be considered as possible delivery vehicles of extraterrestrial organics are asteroids, comets and interplanetary dust particles. The fate of organics should strongly depend on the body's nature, size and thermal history; and the latter is not always clear due to lack of experimental evidences. For comets, catastrophic airbursts with energy dissipation through evaporation of volatile components (mainly water) were suggested to be a scenario where high degree of survival of organics is possible. Because of the established fluffy character of comets and comet dust it is likely that a large fraction of the dust formed after ablation during the entry, even up to sizes $\sim 10^{-5}$

P5.1 continued

m, would be only slightly heated. Organics in asteroids of comparable size have less chances to survive: for example, asteroids as small as 100 m in radius cannot be efficiently aerobraked.

The most abundant source of extraterrestrial matter accreted to the Earth is interplanetary dust particles. Their interiors can be subjected to high temperatures during atmospheric passage, but final fate of a particle strongly depends on its size, velocity and entry angle. Millimeter and submillimeter-size meteoroids are though to be completely evaporated. However, there are some evidences that the particles of 1-10 μm size are subjected to less than 400-500 °C: epsilon-carbide, preserved nuclear tracks, cubic bismuth oxide Bi_2O_3, poorly graphitized carbon and layer silicates found in the particles collected from the stratosphere. Studies of the micrometeoroids retrieved from Antarctic ice melt water evidence that some particles with sizes ca. 100 μm can also survive atmospheric passage without substantial thermal alteration. For 1-5 μm particles, the rate of sedimentation is extremely slow: several years are required to drop from ~1700-km altitude to the stratosphere at 17-19 km. Then for the particles of <1-μm size, this rate should be even lower; due to that they should not be heated at all and reach Earth's surface intact.

Thus, analyzing different space bodies as possible delivery vehicles for extraterrestrial organics one can find the whole spectrum of thermal histories, from no heating to violent impact environments where temperatures can exceed 10,000 °C. Correspondingly, the biomolecules would have from very high to null chances for survival, and it is easy to predict that this will be the case for such extremes as very small particles and asteroids, respectively. However, one can expect that majority of the scenarios, e.g. involving medium-size particles and comets, represent intermediate cases where the heating will not exceed 1000 °C. In the present work we report the results of systematic pyrolytic studies for survivability of some amino acids and nucleic acid bases in the temperature range of 400-1000 °C with 100 °C increments. In majority of the experiments, a possible non-oxidizing primordial atmosphere was simulated by using pure N_2 gas as the pyrolysis medium; CO_2 atmosphere was used for some selected compounds for comparison.

P5.2

EXPOSURE OF AMINO ACIDS IN EARTH ORBIT

F. Boillot[1], B. Barbier[1], A. Chabin[1], D. Chaput[2], O. Hénin[1] and A. Brack[1].

[1] Centre de Biophysique Moléculaire, CNRS, rue Charles Sadron, F-45071, Orléans cedex 2, France.
[2] CNES, 18 avenue E. Belin, F-31055 Toulouse, France.

Delivery of extraterrestrial organic molecules might have contributed to the origin of life on Earth. For instance, the Murchison meteorite contains about 500 organic compounds including nucleic bases and 74 amino acids, 8 of them beeing proteinaceous. Interestingly, micrometeorites could have constituted the dominant source of organic material (Maurette et al., 1998). We have exposed amino acids and peptides and some of their esters to space conditions in order to study their reactivity in space environment when carried by interplanetary grains.

Glycine and L-amino acids, Ala, Val, Leu, Tyr, Asp and Glu, some of their esters (OBzl, ONb, SEt et OMe) and oligopeptides have been exposed to space conditions, free or associated with clays to mimick the mineral component of micrometeorites. Samples were dried to form films on MgF_2 windows (cut off 210 nm) during two flights in Earth orbit (1994, 1997) on ESA-BIOPAN exposure facility fixed outside a Russian Foton satellite.

No racemization of the exposed samples could be detected after the flights. Acidic amino acids and photosensitive amino acids and peptides esters were significatively photolyzed. The degradation was partly reduced by the presence of clay films (montmorillonite). For several active esters (ONb, SEt, OBzl), an important lack of material was observed both in exposed and shaded control cavities. Partial sublimation could be responsable for the loss of material. When the products were mixed with clay surfaces, the losses were strongly reduced. In the presence of clay, some of the activated esters condensed to form di- and tripeptides.

Barbier B., Chabin A., Chaput D. and Brack A.:1998, Planet. Space Sci. **46**, 391-398.
Brinton K.L.F., Engrand C., Bada J.L. and Maurette M.: 1998, Origins Life Evol. Biosphere **45**, 413-424.
Barbier B., Bertrand M., Boillot F., Chabin A., Chaput D., Hénin O. and Brack A.: 1998, Biol. Sci. Space **12**, 92-95.

P5.3

THE SEARCH FOR LIFE ON MARS: THE EUROPEAN STRATEGY

André Brack and the ESA Mars Exobiology Team*, Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, 45071 Orléans cedex 02, France (brack@cnrs-orleans.fr).

The early histories of Mars and Earth clearly show similarities. Liquid water was once stable on the Mars surface, attesting to the presence of an atmosphere capable of decelerating carbonaceous micrometeorites. Therefore, chemical evolution may have been possible on Mars. The most plausible explanation for the results of the Viking 1 and 2 lander analyses was the presence, at the Martian surface, of highly reactive oxidants. The Viking lander could not sample soils below few centimetres and therefore the depth of this apparently organic-free and oxidizing layer is unknown. Martian meteorites show the presence of organic molecules suggesting that organic matter required for the emergence of a primitive life may have been present on the surface of Mars.

ESA Manned Spaceflight and Microgravity Directorate convened Mars Exobiology Team requested to carry out a study of the experimental strategy and the instrumentation necessary to search for indicators of life, especially extinct life, on or within the surface of Mars. A multi-user integrated suite of instruments was designed including i) surface inspection (geology, mineralogy and search for visible surficial microbial macrofossils) and analysis of the environment (radiation budget and oxidation processes) ; ii) subsurface sample acquisition by core drilling ; iii) analysis of oxidants, minerals and organics (elemental and molecular composition, isotopes, chirality) ; iv) macroscopic and microscopic inspection of the subsurface cores to characterize the mineralogy and to search for life's indicators (paleontological, biological, mineralogical).

Beagle 2, the exobiology/geochemistry lander of Mars Express planned to be launched in 2003, could be the first step. Sample preparation will be achieved with a rock grinder/corer on a robotic arm for rock surface grinding and coring and with a self-burying penetrator (mole) for subsurface sampling. The complete integrated package could be mounted on the lander of Mars 2005 Sample Return mission. Final approval of both steps are still pending.

* Clancy P., Fitton B., Hoffmann B., Horneck G., Kurat G., Maxwel J., Ori G., Pillinger C., Raulin F., Thomas N., and Westall F.

P5.5

RECENT ANALOGUE FROM A NON-MARINE FOSSIL MICROBIAL MAT.

Elizabeth Chacon B*, Sergio Cevallos-Ferriz* and Joachim Reitner**

*Departamento de Paleontología, Instituto de Geología, UNAM, Circuito de la Investigación Científica, Apdo. Postal 70-296., Ciudad Universitaria, México D.F. 04510, México

** Institut und Museum für Paläontologie und Geologie (IMPG), Goldschmidtstrasse 3, D-37077, Germany

It is well-known that a large variety of microorganisms are able to form microbial mats in a wide range of modern environments. These microbial communities are considered to represent recent morphological analogues of ancient stromatolites, from which a good biogeochemical characterization is limited by both, diagenesis and by the low fossilization potential of the building organisms, making difficult to evaluate the composition of the original benthic community. However, some studies have confirmed that the application of some geochemical techniques can be used to trace the former presence of specific organisms. In this work we analyze the lipid distribution of a modern continental microbial mat associated with loci of carbonate precipitation, and thus microbialites construction, as well as some morphological correspondance between a fossil and a recent microbial mat.

P5.6

ORGANIC ANALYSIS OF K-T BOUNDARY SEDIMENTS

Ben Fasbinder, George Cooper, Arthur Weber and Ted Bunch
NASA Ames Research Center, Moffett Field, CA 94035 USA

The 250 km diameter Chicxulub Crater was formed ~ 65 myr ago from the impact of a large asteroid (Alvarez et al., 1980). Ir-rich dust and glassy spherules from the impact formed a thin, global layer that is referred to as the Cretaceous/Tertiary boundary or KTB. The impactor is thought to have been similar to carbonaceous meteorites for several reasons including the discovery of carbonaceous meteorite fragments (Kyte, 1998) and unusual amino acids, α-aminoisobutyric (AIB) and isovaline (Zhao and Bada, 1989), at the KTB. However, carbonaceous meteorites are known to contain a much wider variety of organic compounds, some of which (such as AIB) are rare on Earth (Cronin and Chang, 1993). To date, there has been no reported effort to directly compare carbonaceous meteorites to KTB sediments in terms of the many classes of water-soluble organic compounds known to be in carbonaceous meteorites.

In this study we are examining KTB samples from various worldwide sites to further determine if the distribution of water soluble compounds at the KTB is similar to that of carbonaceous meteorites. An example of a comparison is that between a homologous series of organic compounds found in carbonaceous meteorites and the same series in KTB sediments. Sulfonic acids, relatively abundant in the Murchison meteorite and known to be stable compounds (Cooper and Cronin, 1992), are one example of the many homologous series of organic compounds in Murchison (Cronin and Chang, 1993). In this series, all possible isomers of each sulfonic acid at a given carbon number are present (up to C-4) and there is a decrease in abundance with increasing carbon number. This type of distribution is not seen in samples of biological origin and, if present in KTB samples, will serve as another indicator of extraterrestrial delivery and be helpful in identifying the type of impactor. The study employs a variety of analytical techniques including ion chromatography and gas chromatography-mass spectrometry.

Alvarez, L. W., Alvarez, W., Asaro, F. and Michel, H. V. :1980, Science **280**, 1095.
Cooper, G. W., Onwo, W. M. and Cronin, J. R. :1992, Geochim. Cosmochim. Acta **56**, 4109.
Cronin, J. R. and Chang, S. (1993) *In The Chemistry of Life's Origin*, p. 209-258, Eds. J. M. Greenberg et al., Kluwer Academic Publishers, The Netherlands.
Kyte, F. T. :1998, Nature **396**, 237.
Zhao, M. and Bada, J. L. :1989, Nature **339**, 463.

P5.7

STRECKER SYNTHESIS OF AMINO ACIDS, CONDITIONS LEADING TO THEIR PRESENCE IN THE PRIMITIVE OCEAN AND IN CARBONACEOUS CHONDRITES

Laurence Garrel

NASA Ames Research Center, Moffett Field, CA 94035 USA

The evidence for the Strecker synthesis as the origin of amino acids found in the Murchison meteorite is that carbonyl compounds, hydroxyacids and iminoacetic acids, which are also by-products of the Strecker synthesis, have been found in Murchison (Shock 1990, Cronin 1993, Peltzer 1984).

The three-component system of Strecker, NH_3/HCN/RR'CO leads to an equilibrium between the hydroxynitrile (hydroxyacid precursor) and the aminonitrile (aminoacid precursor). At low concentration of ammonia, this equilibrium is shifted to favor the formation of hydroxynitriles (Moutou 1995). In a previous work (Taillades 1998) we have shown, on the basis of kinetic and thermodynamic studies, that the classical base-catalyzed hydration of nitriles is low, not selective, and cannot significantly modify the proportion of α-aminonitriles and α-hydroxynitriles at equilibrium. On the contrary, we found two specific and efficient reactions of aminonitriles which shift the equilibrium in favor of the α-aminonitriles pathway. One of these reactions is the catalysis of the hydration of aminonitriles by carbonyl compounds.

The presence of carbonyls compounds in Murchison (Jungclaus, 1976) suggests that, in the hypothesis of a Strecker synthesis in the meteorite parent body, carbonyl compounds could have been in excess comparatively to the cyanide concentrations. An experimental study of their influence on systems of Strecker, under plausible meteorite parent body conditions, shows that their catalytic effect could have had an infuence on the yield of a Strecker synthesis which may have occured on the meteorite parent body.

Cronin, J.R, Pizzarello, S, Epstein, S and Krishnamurthy, R. V. : 1993, Geochim. Cosmochim. Acta, **57**, p 4745

Jungclaus, G.A. , Yuen, G.U and Moora, C.B. : 1976, Meteorites, **11** n3

Moutou, G., Taillades, J., Benefice-Malouet, S., Commeyras, A., Messina, G. and Mansani, R. : 1995 J. Phys. Org. Chem, **8**, p 721

Peltzer, E.T., Bada, J.L., Schlesinger, G. and Miller, S.L. : 1984, Adv. Space. Res., **4**, n12, p 66

Shock, E. L and Schulte, M.D.: 1990, Geochim. Cosmochim. Acta , **54**, p 3159.

Taillades, J., Beuzelin, I., Garrel, L., Tabacik, V., Bied C. and Commeyras, A.: 1998, Origins of Life and Evolution of the Biosphere, **28**, p 61-77.

P5.8

CARBON ISOTOPIC ANALYSES OF INDIVIDUAL MICROSCOPIC FOSSILS: A NOVEL TOOL FOR ASTROBIOLOGY

Christopher H. House, J. W. Schopf, T. Mark Harrison
Department of Earth and Space Sciences and the Center for Astrobiology, University of California, Los Angeles, CA 90095-1567

Karl O. Stetter
Lehrstuhl für Mikrobiologie und Archaeenzentrum, Universität Regensburg, 93053 Regensburg, Germany

As shown by ion microprobe analyses of individual Precambrian microfossils, measurements of the carbon isotopic composition of ancient microscopic specimens provide promising means to decipher the biochemistry of early life. We have analyzed individual microfossils permineralized in stromatolitic cherts of the ~850 Ma-old Bitter Springs Formation of central Australia and the ~2,000 Ma-old Gunflint Formation of southern Canada.

This preliminary work indicates that reliable carbon isotopic data can be obtained from ancient microfossils with a precision and accuracy of about 1‰ and suggests the presence of isotopic heterogeneity among specimens in the same deposit. The carbon isotopic compositions of the Bitter Springs microfossils support their morphologic-based assignment to the crown group cyanobacteria whereas data for the Gunflint fossils suggest the presence of other microbial groups as well. Work to date demonstrates the feasibility of ion microprobe analyses of individual microscopic fossils and suggests that this technique holds promise for constraining, and perhaps deciphering, the physiology and phylogenetic relations of ancient microorganisms.

This novel technique also may prove applicable to the future study of extraterrestrial samples. Judging from the evolutionary history of life on Earth, it is safe to assume that most and perhaps all ecosystems have at their base a diverse microbe-like component. Thus, extraterrestrial fossils are likely to be in part, and perhaps entirely of microbial size. Study of the isotopic compositions and geochemical signatures of such microfossils could be a powerful tool for inferring the biochemical and physiological characteristics of ancient extraterrestrial ecosystems.

P5.10

IN SITU INORGANIC AND ORGANIC ANALYSIS OF THE MARTIAN SOIL, BY Pyr/CD-GC-MS ON MARS 2005 MISSION

M.Cabane[1], G. Israël[1], P. Coll[1], P. Rannou[1], F. Raulin[2], R. Sternberg[2], A. Jambon[3], E. Chassefière[4], J.-J. Berthelier[5]
1=Service d'Aéronomie, IPSL, Univ. Paris VI, BP102, 75005 Paris, France.
2=LISA 3=Lab. de Pétrologie 4=LMD 5=CETP (all from France)

The most consistent explanation for the Viking failure to detect organic molecules, lies on photochemically produced oxidants, which originate in the atmosphere and diffuse into the regolith, and are a potential source of degradation of organics, including bio-organics. It is important to notice that recently Benner (1998) proposed that some organic polymeric compounds could be present on the Martian surface, and that experimental conditions of Viking's pyrolysis are not compatible with their detection. However, Bullock *et al.* (1993) suggested that Hydrogen peroxide, H_2O_2, produced from atmospheric vapor by UV radiations, may diffuse into the soil before its destruction by the UV. Another source of oxidation could be peroxides produced from the absorbed H_2O (Huguenin, 1979). It is essential to have access not only to surface but also to subsurface samples, to a depth where the possible effect of UV radiation on the chemical indicators of life is negligible, as well as the concentration of oxidizing agents. The knowledge of the quantity of adsorbed H_2O in the soil will enable us to check the models and, joined to the mineralogical analysis, to have a better understanding of the past Martian climate.
To analyze inorganics and organics in the Martian soil, we propose to use the Pyrolysis/Chemical Derivatization-Gas-Chromatography-Mass Spectrometry (Pyr/CD-GC-MS) technique : chemical sensors based on GC and MS instrumentation have already been used in atmospheric probes of surface landers for analyzing extraterrestrial environments, including the analysis of Venus and Mars surface materials. The Aerosol Collector Pyrolyser (ACP) experiment (Israël *et al.*, 1997) uses such technique to analyze the organic aerosols of Titan's atmosphere. In this frame, new instrumentation involving Pyr-GC-MS techniques and using the heritage of Huygens is currently under development for space application, in particular for in situ analysis of cometary nuclei (COSAC and MODULUS experiments on ROSETTA, CHARGE experiment on CHAMPOLLION). We propose then a new experiment, devoted to :

P5.10 continued

IN SITU INORGANIC AND ORGANIC ANALYSIS... (CONTINUED)

• *Search of Inorganic compounds :* as emphasized by the ESA Exobiology-science team (1998), the study of inorganics will allow to deduce a water concentration profile and will give access to water concentration in the deeper layers. This is a key point for studying the possibility of extant life in the present Martian subsurface. The knowledge of the H_2O amount adsorbed in the regolith will permit a better understanding of some aspects of the water cycle. The presence of a duricrust may give information on the vertical migration of salts in the soil and provide some clues on the climatic cycles that occurred during the regolith existence. Carbonate deposits, for example, should be close to the surface if thick atmospheres had been present in late Mars history. Sulfur compounds may be representative of biological processes. It is also essential to measure the abundances of oxidants in the soil, and determine their gradient of concentration with depth, because concentrations of oxidants and of organics are predicted to be anti-correlated.

In opposition to the analysis of the Viking mission, the samples could be heated to temperatures above 500°C, which will permit a reliable CO_2 analysis from carbonates near these temperatures, and other compounds at higher temperatures up to 1100°C. Moreover, the use of detailed temperature steps will permit to assert the temperatures at which H_2O will be emitted, hence an assistance for mineralogical interpretations.

• *Search of Organic compounds* : this search would be of prime importance for exobiology. In the very oxidized Martian environment, the abiotic formation of organics through atmospheric chemical processes is unlikely. The only major abiotic source of organics on the surface and in the near surface is likely extra-Martian importation. Then, by differentiating a meteoritic abiotic origin from a bio-origin, the detection of organic carbon in the Martian soil could provide the evidence of former life processes. The selection of target organics has to take into account the relative chemical stability of the compounds in a highly oxidizing environment. It could be :

- *volatile low molecular weight compounds*
- *medium molecular weight compounds*
- *macromolecular compounds*
- *refractory compounds and thermally fragile compounds of biological or prebiotic interest*

P5.11

LABORATORY REFRACTIVE INDEX MEASUREMENTS OF TITAN'S AEROSOLS ANALOGUES: IMPLICATIONS FOR CASSINI-HUYGENS OBSERVATIONS.

P. Coll[1,2], S.I. Ramírez Jiménez[1,3], J. Lafait[4], R. Navarro-González[3] and F.Raulin[1]
contact: coll@lisa.univ-paris12.fr
(1) LISA, Universités Paris VII and XII, CMC, 94010 Créteil cedex, France.
(2) Service d'Aéronomie du C.N.R.S., 91371 Verrières-le-Buisson, France
(3) Laboratorio de Química de Plasmas y Estudios Planetarios, Instituto de Ciencias Nucleares, UNAM, Circuito Exterior C. U., A. Postal 70-543, 04510 México-City, México.
(4) Laboratoire d'Optique des Solides, Université Paris VI, 75005 Paris, France.

The dissociation of CH_4 and N_2 in the upper Titan's atmosphere environment with low temperature and pressure result mainly from the ultraviolet photons (λ = 121 nm and λ < 100 nm, respectively) with a significant contribution from Saturn's magnetospheric electrons, and also γ rays. The resultant radicals and ions initiate a complex chemistry that ultimately yields to aerosols formation.
Determination of the optical properties of aerosols analogues, with special attention to the real and imaginary parts of their refractive index, have been performed with a laboratory protocol developed at LISA. This protocol avoids oxygen contamination of samples and allows working at mbar pressure and liquid nitrogen temperature domains.
We will discuss the obtained results, of main importance since those of Khare *et al.* (1984), and the implications they have in characterising the haze (main and detached layers) and atmospheric profiles in Titan, and in analysing Cassini-Huygens data.

Khare *et al.*: 1984, Icarus **60**, 127-137.

P5.12

EFFECT OF DIFFERENT ENERGY SOURCES ON A TITAN'S SIMULATED ATMOSPHERE AS A PREBIOTIC MODEL

Sandra Ramírez Jiménez and Rafael Navarro-González
Laboratorio de Química de Plasmas y Estudios Planetarios, Instituto de Ciencias Nucleares, UNAM. Circuito Exterior, C. U. Apartado Postal 70-543, México, D. F. 04510

The transformation from simple organic molecules to more complex ones was a fundamental step in the origins of life and was brought about by the availability of a variety of energy sources on the early planet. Following Miller and Urey's classic work of 1959, there has been several attempts to determine their efficiency in the production of organic compounds in diverse planetary atmospheres. Titan, with its nitrogen dominated atmosphere, offers a natural scenario to simulate planetary scale chemical activity and surface-atmosphere interactions in the absence of life influence (Raulin et al., 1998; Clarke and Ferris, 1997). Nevertheless, the comparative studies carried out so far in simulated Titan's atmospheres lack the quantitative approach (Gupta, et al., 1981), or use unreal atmosphere representations for the production of organics (Scattergood, 1989; Cabane and Chassefière, 1995), making it difficult to assign the correct contribution of each of the different energy sources available in the satellite's atmosphere to the global inventory of detected compounds.

Following the theoretical calculation performed by Sagan and Thopmson (1984), it is evident that UV radiation is the principal energy source in Titan's atmosphere. Depending on the altitude, a single or a band of wavelengths are more or less active. The use of gamma radiation and glow discharge can be used to simulate the second most important effect caused by cosmic rays and Saturninian electrons on the stratosphere. As there has been no detection of lightning activity on Titan at the present time, the lightning discharges can help to simulate the entrance of a high velocity meteors into the atmosphere.

P5.12 continued

EFFECT OF DIFFERENT ENERGY SOURCES

We have added the effect of corona discharges (Navarro-González and Ramírez, 1997) which may developed on methane cloud particles present at tropospheric levels (Griffith et al., 1998).

In an attempt to have the most uniform and representative conditions during the experimentation, a systematic variation of the composition of the simulated atmosphere (5 to 2% CH_4 in N_2), the total pressure (10 to 750 Torr), the irradiation time (some minutes to several hours), and a special control in the identification (use of a GC-MS-IRTF coupled system) and quantification (use of calibration curves) steps, are followed to get precise and accurate energy yields. The following compounds were selected as the most representative and of highest interest prebiotically (Raulin et al., 1998) for this comparison: C_2H_2, C_2H_4, C_2H_6, C_3H_8, HCN, C_2N_2, CH_3CN and HC_3N. Their calculated energy yields will allow us to arrive to confident conclusions when comparing experimental, modeling and observational data.

Cabane, M. and Chassefière: 1995, Planet. Space Sci., **43(1/2),** 47-65

Clarke, D. W. and J. P. Ferris: 1997, Origins Life Evol. Biosphere **27**, 225-248

Griffith, C. A., T. Owen, G. A. Miller and T. Geballe: 1998, Nature **395,** 575-578

Gupta, S., E. Ochiai, C. Ponnamperuma: 1981, Nature **293**, 725-727

Miller, S. L. and H. C. Urey: 1959, Science **130**, 245-251

Navarro-Gonzáles, R. and S. I. Ramírez: 1997, Adv. Space Res. **19(7),** 1121-

Raulin, F., P. Coll, D. Coscia, M. C. Gazeau, R. Sternberg, P. Bruston, G. Israel and D. Gautier: 1998, Adv. Space Res., **22(3),** 353-362

Sagan, C. and W. R. Thompson: 1984, Icarus **59,** 133-161

Scattergood, T. W., C. P McKay, W. J. Borucki, L. P. Giver, H. V. Ghyseghem, J. E. Parris and S. L. Miller: 1989, Icarus **81**, 413-428

P5.13

POSSIBLE ROLE OF VOLCANIC LIGHTNING IN THE PREBIOTIC CHEMISTRY OF EARLY MARS

Antígona Segura and Rafael Navarro-Gonzalez.
Laboratorio de Química de Plasmas y Estudios Planetarios
Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México
Circuito Exterior, C.U., A. Postal 70-543, 04510 México, D.F.

On the early Earth lightning formed in explosive volcanic eruption columns may have been the main source of fixed nitrogen (Navarro-González *et al.*, 1998) and therefore was a favorable environment for prebiotic synthesis (Basiuk and Navarro-González, 1996).

Exploration of Mars has shown that life may have developed about 3.8 billion years ago. Since volcanism was globally distributed (Mouginis-Mark *et al.*, 1992) and highly explosive (Wilson and Head, 1983; Gregg and Williams,1996) during its early history, volcanic plumes could have been a favorable environment for the production of key molecules needed for chemical evolution and the origins of life. A thermodynamic model was used to determine the equilibrium composition of volcanic Martian gases based on SNC meteorites parental magma composition. Preliminary results of the products obtained from this gases under volcanic lightning conditions will be presented.

Basiuk, V. A. and R. Navarro-González: 1996, Origins Life Evol. of the Biosph., 26, 173.
Gregg, T. K. and S. N. Williams: 1996, Icarus, 122, 397.
Navarro-González, R., Molina, M. J. and Molina, L. T.: 1998, Geophys. Res. Lett., 25, 3123.
Wilson, L. and J. W. III Head: 1983, Nature, 302, 663.

P5.14

BIOGENIC ACTIVITY IN SELECTED MARTIAN METEORITES

Everett K. Gibson, Jr[1]., David S. McKay[1], Kathie Thomas-Keprta[2] and Frances Westall[1], [1]SN, NASA-Johnson Space Center, Houston, TX 77058 and [2]C-23, Lockheed-Martin Inc., NASA Rd. 1, Houston, TX 77058.

Criteria are well established within the scientific community for the acceptance of evidence for biogenic activity within samples from the early Earth. The eight criteria are: (i) geologic context, (ii) age and stratigraphic location, (iii) cellular morphology, (iv) colonies, (v) biominerals, (vi) isotope patterns, (vii) organic biosignatures, and (viii) features indigenous to sample. In the case of samples from Mars, we must also apply the same criteria. For the martian meteorite ALH84001, we have presented evidence which indicates possible biogenic activity associated with the 3.94 b.y. old, fracture-bound carbonate deposits (McKay et al., 1996). Subsequent major criticism of our hypothesis concerned the fact that many of the biogenic features could have been introduced during the time the meteorite was in Antarctica, prior to its collection. We address the possibility of Antarctic contamination and compare our evidence with accepted criteria for establishing the presence of past life. Although, we are close to matching some of the required criteria (likely biominerals, organic biomarkers, bacterial appendages, microfossils and indigenous features), there are others (well-documented geologic context, and evidence for colonies) which have not yet been met. It is not yet possible to come to a definitive conclusion concerning life on early Mars, but it is hoped that continued research will provide more relevant information.

P5.14 continued

Biogenic Activity in Selected Martian Meteorites

The Nakhla meteorite was recently shown to contain possible microfossil structures which appear to be more convincing than those observed in ALH84001 (McKay et al., 1999). Nakhla was an observed meteorite fall and is, therefore, not affected by potential Antarctic weathering and contamination effects. Nakhla is only 1.3 b.y. in age and contains residual martian atmospheric gases. Numerous coccoid-shaped possible microfossils between 400 nm and 1.5 micron in size, as well as possible mineralized biofilms are present, within the accepted martian preterrestrial iddingsite/ smectite alteration products. The shapes and sizes are essentially identical to those accepted microfossils from the terrestrial rock record (Westall, 1999). Reduced organic carbon components (up to $\delta^{13}C$= -40‰), including PAHs, are present within the alteration phases (Wright et al., 1998). Inorganic carbonates ($\delta^{13}C$ =+13 to +18‰) are associated with the alteration components (Wright et al., 1998). It is still necessary to determine definitively that these structures are indigenous to the meteorite and were not introduced after collection. If the biogenic features within both ALH84001 and Nakhla are proved to be martian, it demonstrates that Mars had viable biogenic activity over the period 3.94 to at least 1.3 b.y. This is a similar geologic time interval to that observed for development of life on the Earth.

(1) McKay D.S., et al. (1996) Science 273, 924-930
(2) McKay D.S., (1999) 30th Lunar Planet. Sci. Conf. Absts.
(3) Westall F. (1999) J. Geophys. Res., Planets. (in press)
(4) Wright I.P., M.M. Grady, A.F. Gardner and C.T. Pillinger, (1998), 29th Lunar Planet. Sci. Conf., Absts.

P5.15

ELONGATED PRISMATIC MAGNETITE CRYSTALS IN MARTIAN METEORITE ALH84001: EVIDENCE OF BIOGENIC SIGNATURES?

Kathie L. Thomas-Keprta[1], Dennis A.Bazylinski[2], Susan J. Wentworth[1], David S. McKay[3], Mary Sue Bell[1], Everett K. Gibson Jr. [3], Christopher S. Romanek[4], [1] Lockheed Martin, 2400 Nasa Rd. 1, Mail Code C-23, Houston TX 77058, [2] Iowa State University, Dept. of Microbiology, 207 Science 1, Ames, IA 50011, [3] NASA/Johnson Space Center, SN, Houston, TX 77058, [4] Savannah River Ecology Laboratory, Drawer E, University of Georgia, Aiken, SC 29802

Fine-grained magnetite crystals, in the Fe-rich rims of carbonate globules of martian meteorite ALH84001, have been proposed as fossil remains of primitive Martian organisms (McKay et al., 1996). We report observations on the size and shape distributions and chemical compositions of magnetites from ALH84001 and compare them to biogenic and inorganic magnetite crystals of terrestrial origin. Magnetite is found in a wide variety of terrestrial rock types and in meteorites, but none has been reported with the specific and unique characteristics of magnetites produced by various kinds of magnetotactic bacteria. We suggest that magnetite in ALH84001 carbonate globules might be explained by low temperature, inorganic and biogenic processes. A significant fraction of magnetite has a pure chemical composition, unique morphology, and length-to-width ratio that are indistinguishable from a variety of terrestrial biogenic magnetite but distinct from all known inorganic forms of magnetite. Unless an inorganic analog for these crystals is found, the presence of elongated prismatic magnetite crystals associated with the carbonate globules in Martian meteorite ALH84001 must be considered as strong evidence for primitive life on early Mars. Although high temperature mechanisms have been suggested for the formation of whisker-shaped magnetite in ALH84001 (Bradley et al., 1998), we suggest that all ALH84001 magnetite likely formed in the presence of low temperature fluids.

(1) McKay, D.S., E.K. Gibson, Jr., K.L. Thomas-Keprta, H. Vali, C.S. Romanek, S.J. Clemett, X.D.F. Chillier, C.R. Maechling and R.N. Zare (1996) Science **273**, 924-930

(2) Bradley, J.P., H.Y. McSween, and R.P. Harvey (1998) Meteoritics and Planetary Science **33**, 765-773.

P5.16

THE SUBLIMATION AND SURVIVAL OF AMINO ACIDS AND NUCLEOBASES IN THE MURCHISON METEORITE DURING A SIMULATED ATMOSPHERIC ENTRY HEATING EVENT

Daniel P. Glavin and Jeffrey L. Bada
Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA, USA 92093-0212

The delivery of organic molecules by exogenous debris could have played a role in the seeding of the early Earth with pre-biotic compounds necessary for the origin of life (Anders 1989; Chyba and Sagan, 1992). Murchison, a carbonaceous chondrite which landed in southeastern Australia in 1969 has been extensively studied, and is known to contain an abundance of extraterrestrial organic compounds including amino acids and nucleobases (Cronin et al., 1988). Although the effect of heating on amino acids and nucleobases has been studied previously (Cronin and Moore, 1974; Basiuk and Navarro-Gonzalez, 1998), neither of the experiments were representative of the high temperatures associated with frictional heating during meteorite atmospheric entry (Rizk et al., 1991; Rietmeijer 1996).

In this study we report the effects on the amino acids and nucleobases in the Murchison meteorite when heated to a temperature of 1100°C for several seconds under reduced pressure (800 mTorr air). All heating experiments were conducted using a sublimation apparatus designed to sublime and recover amino acids from natural samples at elevated temperatures (Glavin and Bada, 1998). Even though most of the amino acids and nucleobases in Murchison did not sublime after exposure to heat, approximately 40 to 80% of the amino acids in the meteorite still survived with no evidence of thermal decomposition or racemization (Fig. 1). Remarkably, almost 90% of a mixture of nucleobases including adenine (A) and guanine (G), which are both present in Murchison at part per million levels (Van der Velden and Schwartz, 1977), also survived after heating to 1100°C. These amino acid and nucleobase recoveries are much higher than the 1 to 10% values previously estimated (Basiuk and Navarro-Gonzalez, 1998). Our results suggest that a large fraction of these organics in carbonaceous chondrites will survive the frictional heating associated with atmospheric entry with minimal thermal degradation.

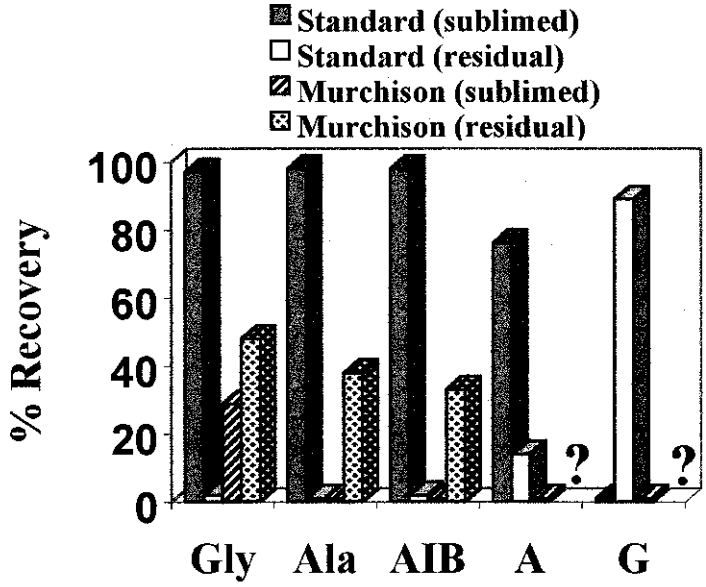


Figure 1. A comparison of the amino acid and nucleobase recoveries in the sublimed and residual extracts from a standard and the Murchison meteorite sample after heating at 1100°C for several seconds.

Anders, E.: 1989, Nature **342**, 255.
Basiuk, V. A. and Navarro-Gonzalez, R.: 1998, Icarus **134**, 269.
Chyba, C. F. and Sagan, C.: 1992, Nature **355**, 125.
Cronin, J. R., Pizzarello, S. and Cruikshank, D. P.: 1988, *Meteorites and the Early Solar System*, University of Arizona Press, Tucson, p. 819.
Cronin, J. R. and Moore, C. B.: 1974, Meteoritics **9**, 327.
Glavin, D. P. and Bada, J. L.: 1998, Anal. Chem. **70**, 3119.
Rietmeijer, F. J. M.: 1996, Meteoritics **31**, 237.
Rizk, B., Hunten, D. M. and Engel, S.: 1991, J. Geophys. Res. **96**, 1303.
Van der Velden, W. and Schwartz, A. W.: 1977, Geochim. Cosmochim. Acta. **41**, 961.

P5.17

CHARGE SEPARATION AND ELECTROSTATIC INTERACTIONS IN THE SOLAR NEBULA

Glenn E. Ciolek and Wayne G. Roberge, Rensselaer Polytechnic Institute

Dust and magnetic fields are ubiquitous throughout the interstellar medium, and are also generally considered to be important constituents of protostellar/protoplanetary disks. We examine the interaction of magnetic fields and dust grains under conditions designed to simulate the protosolar nebula. In particular, we investigate the possible separation of charged grains as a result of hydromagnetic shocks in the weakly ionized solar nebula. An effect of such a charge separation is that streaming between grain species of different sizes and charge distributions may have occured, which gave rise to electrostatic disturbances or instabilities that were amplified and grew in the gas behind the shock. Such a process may have led to the onset of energetic discharges and contributed to the formation of chondrules. Moreover, the local heating resulting from this phenomenon may also have affected chemical processes that occurred in the reducing nebular environment.

P5.18

"ORIGINS OF LIFE: FROM INTERSTELLAR MOLECULES TO INTRONS". NEW YORK CENTER FOR STUDIES OF THE ORIGINS OF LIFE A NASA SPECIALIZED CENTER OF RESEARCH AND TRAINING (NSCORT).

James P. Ferris
Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180

An NSCORT was established at RPI, SUNY Albany and the College of St. Rose in the Capitol District of NY in July 1998. The scope of the research encompassed in the title of the grant will be outlined in the poster.

The investigators in the NSCORT and their areas of research are:

Douglas Whittet, Astrophysics, RPI - Spectroscopic studies of interstellar/protostellar organic matter using astronomical observations from ground-based and satellite observatories.

Wayne Roberge, Astrophysics, RPI - The chemistry of the early solar nebula, emphasizing the possible role of shock-driven reactions.

Michael Gaffey, Earth and Environmental Sciences, RPI - Surface mineralogies derived from analysis of reflectance spectra will be used to determine the aqueous and thermal alteration conditions within asteroids in order to constrain the production and alteration of prebiotic organic molecules in these bodies.

John Delano, Earth and Atmospheric Sciences, SUNYA- The role of cometary, meteoritic and asteroidal impacts on the origin of life and the outgasing of reduced organics from the mantle of the primitive Earth.

James P. Ferris, Chemistry, RPI- The clay-mineral catalyzed synthesis of RNA and photochemical transformations in the atmosphere of the primitive Earth.

William J. Hagan, Jr., College of St. Rose- Prebiotic phosphorylation reactions.

Sandra Nierzwicki-Bauer, Biology, RPI - The search for molecular fossils of the RNA world; studies of subsurface microorganisms for the presence of introns; community structure of microbial mats.

For information about our research and outreach programs see the NSCORT web site at http://www.rpi.edu/dept/phys/Astro/origin.html

P5.19

ASTEROIDS AS SOURCES OF PREBIOTIC COMPOUNDS

Michael J. Gaffey, NY Center for Studies on the Origin of Life, Dept. of Earth and Environmental Sciences, Science Center, Rensselaer Polytechnic Institute, 110 8th Street, Troy, New York 12180-3590 USA.
e-mail: gaffem@rpi.edu

Two sources have generally been invoked to provide the prebiotic compounds necessary for the origin of life: (a) production in a reducing environment in the Earth's early atmosphere and/or hydrosphere, and (b) delivery of extra-terrestrial organics to the early terrestrial environment. In the second scenario, comets have been identified as the primary potential extra-terrestrial source because of a high abundance of carbon compounds. Asteroids, largely because of their significantly lower carbon content, have generally been ignored as potential sources of prebiotic compounds to the early Earth. Considerations of the delivery mechanisms for cometary and asteroidal materials requires reconsideration of this assumption.

Both asteroids and comets contain organic compounds. In comets, these include interstellar molecules and those formed by nebular processes. In asteroids, these include similar molecules plus compounds formed by aqueous reactions within their parent bodies, as evidenced by the organics in carbonaceous chondrite meteorites. The low temperature formation conditions of comets provided a better environment to preserve interstellar and nebular organics, while the warmer formation and post-formation conditions of asteroids destroyed many of the pre-existing organics but also led to the formation of additional organic species. The abundance of carbon compounds in comets is approximately ten times their abundance in the most carbon-rich asteroids. Hence the preference for comets as a source of any extra-terrestrial prebiotic compounds.

However, a selection effect operates to redress this imbalance. To provide prebiotic compounds to the early Earth, the extra-terrestrial fragments must survive delivery to the terrestrial environment. Objects which enter the atmosphere at high velocities are vaporized, a process which will destroy any pre-existing organic molecules. Based upon meteorite and meteor studies, 30 km/sec is the effective upper limit for any portion of the meteoroid to survive in an unaltered state. For very large high-velocity projectiles, entry vaporization does not effect a significant fraction of the mass, but impact heating will vaporize the projectile. The average entry velocity of material from cometary orbits is >50 km/sec, with only a very small fraction below 30 km/sec. Average entry velocities from asteroidal orbits is <26 km/sec, with a substantial fraction below 20 km/sec. Although cometary debris are much richer in potential prebiotic molecules, very little of this material can survive atmospheric entry. Considering the selection effect for lower entry velocities, asteroids are probably at least as important as an extraterrestrial source of the precursors of life as are comets.

P5.20

ORGANIC MOLECULES IN INTERSTELLAR ICES STUDIED WITH THE INFRARED SPACE OBSERVATORY

Douglas Whittet, Erika Gibb and Albert Nummelin
New York Center for Studies of the Origins of Life, and Department of Physics, Applied Physics and Astronomy, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

A clear understanding of the chemical composition and evolution of interstellar matter in regions of active star formation is fundamental to our quest to understand the origin and early evolution of the solar system and of Earth. Volatiles as well as refractory minerals may survive the formation process to become incorporated into planetesimals. Asteroids and comets are known to contain organic matter, and they may have been a rich source of both water and prebiotic molecules on Earth and other planets during the later stages of accretion.

We are developing a comprehensive inventory of organic molecules in interstellar ices. This has become a realistic goal only recently, with the launch of the Infrared Space Observatory (ISO) by the European Space Agency in 1995: ISO provides a facility for infrared spectroscopy unhindered by telluric absorption, covering the entire spectral range of vibrational modes in solids of exobiological interest. Interstellar molecules detected in the solid phase to date include H_2O, CO, CO_2, CH_3OH, CH_4, H_2CO, OCS and HCOOH. As data reduction techniques applied to the ISO data continue to be refined, we will probe not only the major features of abundant constituents but also the second-order features that contain much detailed information on lesser, but potentially vital, carbon-bearing molecules. Recent observations of comets are leading to parallel advances in our understanding of their chemical compositions. Detailed comparisons are therefore possible for the first time.

Our inventory of abundances for carbon-bearing molecules in interstellar and protostellar ices will cover all species available to investigation by infrared spectroscopy down to levels of 1% or less. This database will clearly be an important resource for future work in exobiology, leading not only to a better understanding of the cosmic history of the biogenic elements in the interstellar medium, but also to new constraints on chemical models of the early solar system and of the Earth during the late accretion phase.

P5.21

A POSSIBLE EUROPA EXOBIOLOGY

Antonio de Morais
Institute of Physics, USP University, São Paulo, SP 05346-000, Brasil

The Near Infrared Mapping Spectrometer (NIMS) of the NASA's Jet Propulsion Laboratory's Galileo spacecraft is being used to study the atmospheric and surface composition of Jupiter and its moons, as Galileo orbits that planet. The NIMS covers the wavelength (λ) range 0.7 to 5.2 μm with up to 408 spectral channels and a resolving power of 40 to 200 ($\lambda/\Delta\lambda$). The instrument's instantaneous field of view is 0.5 mrad, giving a spatial resolution (pixel size) of 5 km at 10,000-km distance. The spectra are calibrated to units of reflectance at the specific geometry of the observation compared with the reflectance of a perfectly diffusing (Lambert) surface with the use of the solar spectrum and a combination of ground and in-flight NIMS measurements of calibration targets.

Here in this paper, I propose NIMS to be used to look for hydrated phosphorus salt minerals in the optically darker (lower visible albedo) areas of Europa's icy surface, including the lineaments and spots; I also suggest a biochemical model for possible microbial life inside Europa.

Europa is the second Galilean satellite outward from Jupiter. It is a lunar-sized object with a Fe-FeS central core, a silicate mantle and a water-ice surface of relatively young geologic age exposed to vacuum.

NIMS reflectance spectra for Europa's nonicy dark regions show the presence of hydrated sulfate and carbonate salt minerals, such as epsomite ($MgSO_4 \cdot 7H_2O$) and natron ($Na_2CO_3 \cdot 10H_2O$) forming the material composing those portions of the surface, which are evaporite deposits formed by water, rich in dissolved salts, reaching the surface from a liquid water-rich layer (seas) underlying the ice crust of Europa. The darker terrains possess visible reddish and yellowish hues (phosphorus exibits such color characteristics), and the mixtures of carbonate and sulfate minerals provide good matches to Europa's nonice spectrum. So, I surmise phosphorus minerals, in the form of hydrated phosphate salts mixtured with the other salts could give a better match to Europa's nonice spectra.

If this turn to be correct, the presence of phosphorus salts within Europa increases the possibility Europa's internal seas (with hydrothermal activity and circulation of those biochemical important ions) could be an

environment with potential for sustaining microbial life, since phosphorus is a biological fundamental element (as carbon, nitrogen and sulfur).

Comets impacts onto Europa, driven by the Jupiter's gravitational field, delivered great quantities of those biological fundamental elements to Europa, during its formation era (inumerous impact craters are visible on the surface of other Jupiter's moons).

The five combined fundamental conditions for the origin of life, as we know it for prebiotic primitive Earth, e.g., the existence of heat (gravitationally driven via internal tides by Jupiter) and liquid water (beneath the crust) plus organic molecules in Europa, metallic compounds - some metals as iron, magnesium and tungsten are fundamental for bacteria metabolisms - and light ametals (sulfur, and phosphorus if present) give Europa the potential for sustaining microbial life.

The existence of thermal energy and possible liquid water in Europa, for a period of time long enough for the formation of biomolecules (aminoacids, proteins, lipids, polysaccarids, polynucleotides) with capacity of survival, protocells and efficient catalytical metabolisms is fundamental for biochemical evolution. The capacity of survival and efficient catalysis is directly related to the principle of least energy possible.

One can consider that the rate state of biogeochemical evolution is directly related to the production of heat inside a planetary body with liquid water. Since Europa's internal thermal energy production is $1/10^{th}$ of Io and Io's heat is twice of Earth, then Europa's production of heat is $1/5^{th}$ of the Earth. So, the present state of biogeochemical evolution of Europa possibly can be analogue to that on Earth 3.6 billion years ago, since Earth and Europa were formed 4.5 billion years. At that time on Earth there were simple procarionts (mycells and bacteria). Today, inside Europa, near the possible hydrothermal volcanic vents throats, the mixture of hot and colder water and polypeptides can have formed sheets of gelatinous, jellyfish-like, material (formed by catalytic proteinoid microspheres) containing several chains and agglomerations of archaebacteria. A future NASA's robot-microsubmarine mission being planned to explore the interior of Europa could be targeted to go towards the sea bottom near volcanic vents to examine the existence of those primitive microorganisms.

McCord, T. B., *et al*:1998, Science **280**, 1242.
Anderson, J. D. *et al*:1997, Science **276**, 1236.
Libes, S. M.:1992, *An Introduction to Marine Biogeochemistry*, John Wiley & Sons, Inc., New York, p. 301.

P5.22

POLYOLS IN CARBONACEOUS METEORITES

Novelle Kimmich, George Cooper and Katrina Brabham
NASA Ames Research Center, Moffett Field, CA 94035 USA

The Murchison meteorite is the most studied carbonaceous meteorite and has been shown to contain several classes of pre-biotic organic compounds (Cronin and Chang, 1993). Sugars and other polyols, possibly the most interesting compounds in terms of the study of the origin of life, have not been reported to occur in Murchison. Because of the important role of ribose, deoxyribose, glycerol, and other polyols in contemporary biology, the search for a pre-biotic source of polyols is of great interest.

A likely abiotic mechanism of producing polyols, the "Formose" reaction (Langenbeck, 1956), simply requires formaldehyde in aqueous solution. Formaldehyde is widespread in the interstellar medium and comets and therefore could have undergone aqueous condensation reactions in meteorite parent bodies. Meteorites and comets have delivered organic matter to the Earth and other planets since the formation of the solar system. If polyols are indigenous to meteorites (and comets) they could have been part of the initial mixture of pre-biotic compounds that led to life on the early Earth.

In this study we extended the search for polyols from Murchison to other carbonaceous meteorites including Murray, which also contains several soluble organic compounds. Techniques such as HPLC with light scattering detection, gas chromatography-mass spectrometry, and gas chromatography-isotope ration mass spectrometry, allow the detection and characterization of a range of polyols. Results of isotope ratio measurements and molecular analysis will be presented.

Cronin, J.R. and Chang, S. (1993) *In The Chemistry of Life's Origin*, p. 209-258, Eds. J.M. Greenberg et al., Kluwer Academic Publishers, The Netherlands.
Langenbeck, W. (1956) J. Prakt. Chem. **(4) 3**, 196-210.

P5.23

GROWTH OF METHANOGENS ON A MARS SOIL SIMULANT IN A WATER-STRESSED ENVIRONMENT

Timothy A. Kral and Curtis R. Bekkum, Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA 72701

Even though the Viking Lander missions in 1976 found no substantial evidence of life on the surface of Mars, investigators continue to believe that life may exist in subsurface habitats. A subsurface source of molecular hydrogen (or possibly carbon monoxide), along with carbon dioxide (which is already abundant in the martian atmosphere), and liquid water, might support the growth of chemoautotrophic methanogenic microorganisms. Because nutrient requirements for many methanogens are so minimal, the question can be asked as to whether any methanogenic species can grow in an aqueous environment where martian soil supplies all required nutrients except for the hydrogen and carbon dioxide. Also, if growth occurs under these conditions, what is the least amount of water required to sustain growth? In experiments designed to answer these questions, *Methanosarcina barkeri, Methanobacterium wolfei, Methanobacterium formicicum,* and *Methanococcus maripaludis* were grown in standard media, centrifuged, and washed three times with a carbon dioxide-saturated sodium hydroxide buffer solution. The cell pellets were suspended in the same buffer and then varying volumes of each were anaerobically added to sealed tubes, each containing 5g of Mars soil simulant in a carbon dioxide and hydrogen atmosphere. The Mars soil simulant already contained approximately 20% water, so the total amount of water present was greater than the volume added. The total amount of water in each tube varied from less than saturating to standing liquid. Each culture tube was then incubated at the optimal temperature for the respective organism. Results indicate that the Mars soil simulant contains sufficient nutrients to support growth (as determined by methane production) of *M. wolfei, M. formicicum*, and *M. barkeri. M. maripaludis* has shown no growth on Mars soil simulant. *M. wolfei* shows methane production when as little as 2 ml of cell suspension is added to the 5 g of Mars soil simulant. *M. barkeri* grows fairly well when only 1 ml is added. A small amount of methane is even evident with the addition of 0.5 ml. *M. formicicum* grows more slowly and requires the addition of at least 3 ml of cell suspension. For the faster growing *M. wolfei* and *M. barkeri*, under conditions where standing liquid was present (when volumes greater than 3.5 ml are added), growth was comparable to that seen in standard growth media. Under conditions where there was no standing liquid, from less than saturated to saturated, growth rate correlated with the amount of water. Thus, even with small amounts of liquid water available below the surface of Mars, certain methanogens might be able to survive.

P5.24

AN UNDERGRADUATE, NON-MAJORS COURSE IN ASTROBIOLOGY

Bruce M. Jakosky
LASP/University of Colorado, Boulder, CO 80309-0392

We have created an upper-level, non-science-majors course in astrobiology. The course, like the field, deals with understanding the origin and evolution of life on Earth, the environmental conditions required for life to originate and to exist, where in our solar system those conditions might be met, the discovery of extrasolar planets and the potential for habitable Earth-like planets, the potential for intelligent life elsewhere, and the philosophical and societal issues connected to life elsewhere. As such, it brings together results from the otherwise disparate fields of geology, biology, planetary science, and astrophysics, and reaches out into the humanities as well through the philosophical and sociological issues. Students who take the course come from all parts of the campus, including the physical sciences, biological sciences, humanities, engineering, and even the business school. Because of the widespread interest in the topic, it provides a vehicle for teaching students about the nature of science, the connections between science and society and between science and religion, and the degree of validity of scientific thought. In particular, it provides a way to show that the world around us can be understood by observing it. Our course is cross-listed between two different departments, and has been oversubscribed (with a limit of 75 students) for two semesters in a row.

P5.25

PHILOSOPHICAL ISSUES CONNECTED TO THE SEARCH FOR LIFE ELSEWHERE

Bruce M. Jakosky
LASP/University of Colorado, Boulder, CO 80309-0392

Recent developments in biology, geology, planetary science, and astrophysics have brought excitement in the potential for life elsewhere to a high level. One of the less-often-discussed aspects of the resulting search concerns why we are interested in the potential for life elsewhere, what the philosophical issues are that drive us to search, and what it would mean to find (or to not find) convincing evidence for extraterrestrial life. That such a large fraction of the public is interested in the issues and that much of the research in the planetary and astrophysical aspects of astrobiology has few practical applications, yet enjoys widespread support regardless, underscores the deep meaning of the results. This likely connects up to the value of exploration in our society, to the desire to understand our origins and how we as a species and as a society fit into the world around us. That is, it connects to understanding what the nature of humanity is and what it means to be human. We as a society have been exploring the world around us for more than 2000 years, and, in fact, that exploration arguably is the hallmark of civilization. These issues will be discussed, along with the connections between science in general and society and the religious aspects of extraterrestrial life.

P5.26

THE LATE HEAVY BOMBARDMENT OF THE MOON – DID IT LEAVE ANY TRACES ON EARTH?

Aivo Lepland & Gustaf Arrhenius
Scripps Institution of Oceanography, University of California San Diego, La Jolla CA, USA 92093-0220

The occurrence of biologically fractionated carbon in the early Archean 3.86 Ga Akilia and 3.8 Ga Isua metasedimentary rocks (Schidlowski, 1988; Mojzsis et al., 1996; Rosing, 1999) have indicated the existence of life already at the very bottom of the Earth's stratigraphic record. The accumulation of the Akilia and Isua sediments partly overlapped with the period of the lunar late heavy bombardment with effects which, as often assumed, would have left Earth uninhabitable well past 3.8 Ga. Still, related impact events have not left any obvious traces in the metasediment sequences so far investigated.

Detailed stratigraphic study of meter to submillimeter-scale banding and lamination of Isua banded iron formation (BIF) using several hundred meter long drill cores has been undertaken in order to search for impact effects. The alternating magnetite and quartz laminae in the chemically precipitated BIF reflect the changes in circulation and reactant supply of currently unknown periodicity in the primary sedimentary environment. Evidence of major impacts could be present in the sediment sequences as a coarse grained, unsorted surge deposits, formed by the resulting giant tsunami waves. Enhanced influx of cosmic debris would also be recorded by sediment layers enriched in the platinum group metals. However, a straightforward interpretation of extraterrestrial influence is complicated by the amphibolite grade metamorphic overprint, modifying the original textural and mineral character of the sediments. By compensation for the diagenetic and metamorphic effects an attempt is made to reconstruct the composition of the original sediment, permitting conclusions about the conditions in the sedimentary environment. A four-stage model is proposed, attempting to characterize the history of the Isua BIF.

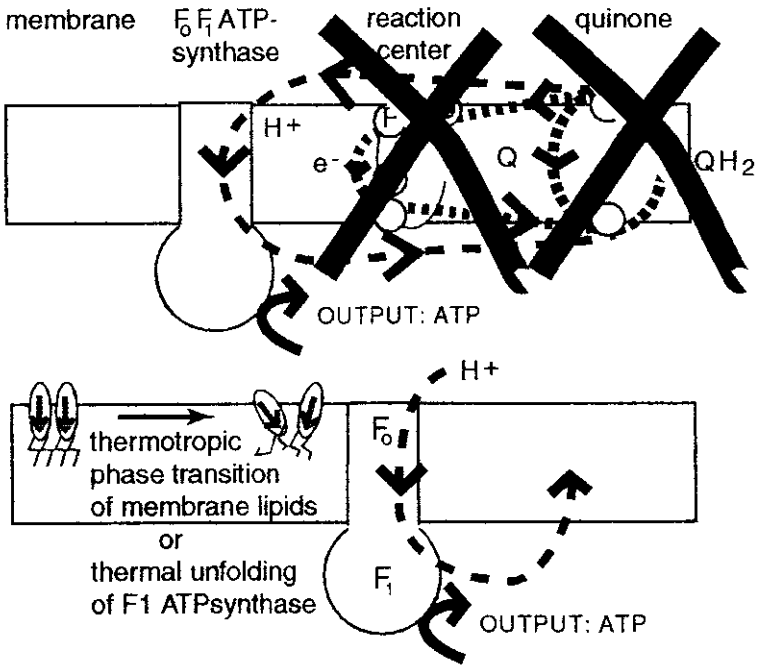
Mojzsis, S.J., Arrhenius,G., McKeegan,K.D., Harrison,T.M., Nutman, A.P.: 1996, Nature **384**, 55-59.
Rosing, M.: 1999, Science **283**, 674-676.
Schidlowski, M.: Nature **333**, 313-318.

P5.27

THERMOSYNTHESIS NICHES IN THE SOLAR SYSTEM

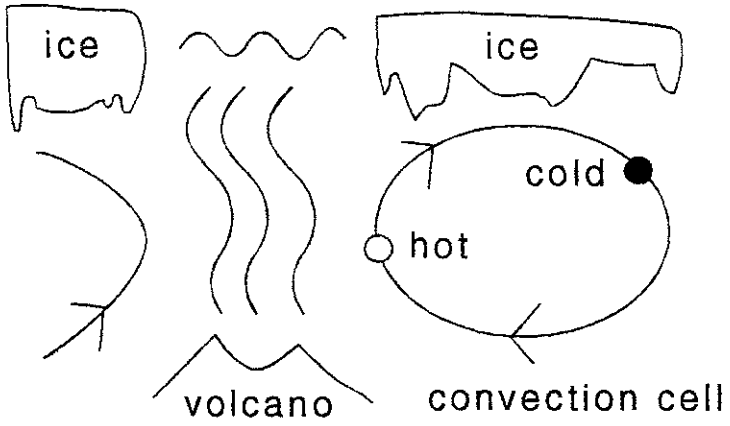
Anthonie W.J. Muller, Dept of Biomedical Sciences,
University of Edinburgh

In all living organisms, including the most simple bacteria, ATP is the energy source for almost all metabolic processes. During respiration and photosynthesis ATP is synthesized by the chemiosmotic mechanism, which uses a complex machinery comprising several proteins and other biomolecules embedded in a biomembrane. Curiously, removal of some components results in a machinery that could, in theory, synthesize ATP *during thermal cycling*: microorganisms could therefore live, or may have lived, without food or light. The mechanisms make use of the well known temperature-induced conformation changes of proteins or of phase transitions of membrane lipids. Biological heat engines (*thermosynthesis*) could operate in convection cells such as in volcanic hot springs, where everything carried along by the convection currents is thermally cycled.



P5.27 continued

THERMOSYNTHESIS NICHES



The power of thermosynthesis is much smaller than the power of photosynthesis, but it enables organisms to live in environments that until now have been considered uninhabitable due to the absence of sunlight. In the Solar System convection must occur where natural waters are heated from below and cooled from above: (1) the ocean on the Jovian moon Europa; (2) water underneath ice shaded from the sun in craters on Mercury and the Moon; and (3) subterranean aquifers on Mars. Thermosynthesis could even occur in (4) meteorites and comets, since rotation in the sunlight must result in thermal cycling of the surface (in the 60s a similar power source was proposed for artificial satellites).

Because of its simplicity, thermosynthesis is a plausible energy source for the first organisms. Wherever they emerged in the Solar System, ejecta from meteorite impacts can have transported them to other planets and their moons. On Earth, thermosynthesizers may have lost the competition with photosynthesizers, but explorers of the Solar System should be aware that they still may be extant elsewhere.

Muller, A.W.J.: 1995, Prog. Biophys. Mol. Biol. **63**, 193
Muller, A.W.J.: 1996, Essays Biochem. **31**, 103
Muller, A.W.J.: 1998, in *Uroboros, or biology between mythology and philosophy*, Arboretum, Wroclaw, p. 139
Muller, A.W.J.: *The Thermosynthesis Home Page*.
http: //www.ed.ac.uk/~awjm
Margosian, P.M.: 1965, *Parametric study of a thermoelectrostatic generator for space applications* NASA Techn. Notes TN D-2763

P5.28

ABIOGENIC CONTINUITY DURING THE AGE OF THE LARGE IMPACTS IN THE EARLY EARTH - SIMULATION EXPERIMENTS

Vicente Marcano, Pedro Benitez, Leticia Miranda and Ernesto Palacios-Prü. Electron Microscopy Center, University of the Andes, P. O. Box 163, Mérida, Venezuela. E-mail: prupal@ing.ula.ve

It is frequently stated that impacts by large bodies in the early Earth could have caused massive destruction of prebiotic organic compounds and sterilization of the Earth, thereby inhibiting abiogenic activity during the Hadean (pre-3800 Myr ago) and the Early Archean (3800 to 3600 Myr ago) ages (Maher & Stevenson 1988, Sleep et al 1989, Chyba 1993, Zahnle & Sleep 1997). However, based on experimental data, we have demonstrated a high probability of the occurrence of a continuous process of abiogenesis on the surface of the oceans during the age of the large impacts (4200-3600 Myr ago) which includes the synthesis of polypeptides, as a result of the heating of the primordial layer of oil, coal tar or tholins probably covering the oceans (Lasaga et al. 1971, Sagan & Khare 1971, 1979, Cleaves & Miller 1998).

Polypeptides were obtained by heating a mixture of four amino acids (DL-glycine, DL-aspartic acid, DL-histidine and DL-asparagine) in heavy mineral oil and in several temperature ranges (140-360°C). We utilized heavy mineral oil as a model of tholins (Sagan & Khare 1979) or primordial oil slick (Lasaga et al. 1971) according to Deamer et al. (1989) and Cleaves & Miller (1998). Assuming a near-total lack of sunlight for extended periods after the impacts (Maher & Stevenson 1988), the experiments were carried out in absence of light in a dark room. Three types of prebiotic atmospheric models were selected for these experiments: $H_2O + CO_2$, 2 NH_3 + H2O and $H_2O + NH_3 + CO_2$. Mixture of gases in oxidizing and neutral atmospheres have equimolar concentrations. The extracted products were fractionated chromatographically on Sephadex G-25-80, thereafter, the resulting fractions were analyzed by spectrophotometry comparing their absorbance spectra with Substance P as control. The peptides obtained were quantified using the modified Lowry protein assay. The molecular weights were estimated by Sephadex gel permeation chromatography and SDS-polyacrylamide gel electrophoresis, using 18% concentration following method of analysis of proteins by SDS-PAGE.

Mineral oil subjected to different temperatures between 140 and 360°C, yielded peptide products between 2.2 and 37%, according to Lowry protein assay. Products obtained, free of compounds not soluble in water by chloroform/hexane extraction, showed a brownish pigmentation, which became darker with the increase of peptide concentration. Fractions obtained from chromatography by Sephadex gel permeation showed important absorbances at 200 nm, which are similar to that obtained for the control polypeptide. Fractions II and III have absorbances near the region of 400 nm

P5.28 continued

probably associated with the brownish pigment, whereas fraction I has no absorbance above 300 nm and lacks visible brownish pigment. Electrophoretic analysis showed three bands from the crude material revealing the presence of peptides with several molecular weights, viz. 2,000, 11,000 and 13,000 daltons. The yield of peptides under 2 NH_3 + H2O atmosphere and temperatures between 240 and 260°C was 21%, whereas at lower temperature range, between 140 and 160°C, the yield was 12%. In an atmosphere model with CO_2, NH_3 and H_2O, we obtained a yield of peptides up to 37%. These proportions are higher than those obtained in a reducing atmosphere. In an atmosphere model including CO_2 and H_2O we obtained a yield of 13.2% between 140-160°C, and 15.6 % between 240-260°C. Results from simulation experiments showed higher yields of peptides in the range 240-260°C and less yields in 140-160°C and 340-360°C ranges.

The abiogenic synthesis model here proposed could have occurred in the hydrothermal spring systems of the mid-ocean ridges of the early Earth. These events could have also occurred in Mars where the existence of oceans covered by oil slick or tholins (Lasaga et al. 1971, Khare & Sagan 1973, Sagan & Khare 1971, 1975), high concentrations of CO_2 and H_2O in its atmosphere and surface (Squyres & Kasting 1994, Carr 1996), large impacts of meteorites (Wetherill 1985) and an intense hydrothermal activity (Squyres & Kasting 1994) would have happened during the first half billion years of its history.

Carr, M. H.: 1996, *Water on Mars*, Oxford University Press, New York.

Chyba, C. F.: 1993, *Geochim. Cosmochim. Acta* **57**, 3351.

Cleaves, H. J. and Miller, S. L.: 1998, *Proc. Natl. Acad. Sci. USA* **95**, 7933.

Deamer, D. W., Harang, E. A. and Seleznev, S. A.: 1989, *Origins of Life Evol. Biosphere* **19**, 291.

Khare, B. N. and Sagan, C.: 1973, *Icarus* **20**, 311.

Lasaga, A. C., Holland, H. D. and Dwyer, M. J.: 1971, *Science* **174**, 53.

Maher, K. A. and Stevenson, D. J.: 1988, *Nature* **331**, 612.

Sagan, C. and Khare, B. N.: 1971, *Science* **173**, 417.

Sagan, C. and Khare, B. N.: 1975, *Science* **189**, 722.

Sagan, C. and Khare, B. N.: 1979, *Nature* **277**, 102.

Sleep, N. H., Zahnle, K. J., Kasting, J. F. and Morowitz, H. J.: 1989, *Nature* **342**, 139.

Squyres, S. W. and Kasting, J. F.: 1994, *Science* **265**, 744.

Wetherill, G. W.: 1985, *Science* **228**, 877.

Zahnle, K. J. and Sleep, N. H.: 1997, in *Comets and the Origin and Evolution of Life*, eds. Thomas, P. J., Chyba, C. F. and McKay, C. P. (Springer, New York), p. 175.

P5.29

RESPONSE OF A *FUSARIUM* SPECIES TO EXTREME CONDITIONS - RESULTS FROM EXPERIMENTS IN LABORATORY

Vicente Marcano, Pedro Benitez, Zulma Peña, Leticia Miranda de Contreras and Ernesto Palacios-Prü. Electron Microscopy Center, University of the Andes, P. O. Box 163, Mérida, Venezuela. E-mail: prupal@ing.ula.ve

The growth of microorganisms under such extreme environmental conditions as exposure to UV radiation, low or high temperatures, absence of oxygen, minimum or no water supply, and very low pH has been known for many years. This fact motivated the development of studies under *in vitro* conditions similar to those of the early earth and extraterrestrial environments during the last two decades. Currently, it is well known the capacity of some microorganisms to support extreme conditions in exobiological experiments of spatial simulation (Dose & Klein 1996) and out of the earth atmosphere (Horneck 1993). Most of these studies have, however, been done using prokaryotes, especially spore-forming bacteria belonging to the genus *Bacillus*, viz. *B. subtilis*.

This work describes the conditions of extreme biological adaptation of an eukaryotic organism that grows in hydrocarbons. The species termed tentatively *Fusarium alkanophilum* V. Marcano & Palacios-Prü was fortuitously found growing on a choleoptera immersed in kerosene. It is able to grow in and degrade several hydrocarbons with minimum oxygen and water requirements, showing a mean growth rate of 2.4 mm per hour. These hydrocarbons include light (kerosene, gasoil) and heavy (mineral oil, paraffin and asphalt) compounds. This species is also able to grow on hydrocarbon media with potato dextrose agar (PDA), malt extract-agar (MA) and modified Czapek (CZA) showing several phenotypes. However, the growth of *F. alkanophilum* was inhibited in $< C_{10}$ hydrocarbons viz. toluene, dioxane, hexane, benzene. *Fusarium alkanophilum* showed no growth on kerosene media with 0.1-5.0 % creosote. No growth was also observed in synthetic media containing urea, ammonium carbonate and potasium ferrocianurum as nitrogen sources in the presence of hydrocarbons. The organism is proteolytic, since it grows in culture media of hydrocarbons containing albumin, glycoprotein and gammaglobulin as source of carbon and nitrogen, however, in media containing other proteins that lack sulphur linkages (e.g. casein, papaine, trypsin), no growth was observed. In cultures with kerosene and PDA having H_2O_2, between 0.2-0.6 µl/ml, notable growth was observed, whereas in higher concentrations of H_2O_2, the growth was inhibited. In simulation experiment under conditions of 354 nm UV-radiation, low pressure (-1 atm), NH_3 vapour and mineral oil in PDA, *F. alkanophillum* showed an active growth, whereas in 254 nm UV-radiation and asphalt, growth was normal. In another experiment having 254 nm UV-radiation, CO_2 and

P5.29 continued

mineral oil in PDA, *F. alkanophillum* showed enhanced growth. Moreover, it showed optimum growth in different hydrocarbons even in the absence of light. Spore formation was stimulated markedly by exposure to 354 nm UV-radiation. Optimal growth occurs at 25°C; on mineral oil and kerosene plates, an increase or decrease of 10°C seriously affected growth rate, while above 40°C, growth was inhibited. Immersion in mineral oil at all temperatures, from -20°C to 55°C, during 15 days had no deleterious effects on the subsequent growth of the fungus from germinated spores, but immersion in kerosene at 55°C during 15 days fully inhibited spore development.

Analysis by TEM of *F. alkanophillum* grown in light hydrocarbon media revealed the presence of a cell wall, however, the absence of plasmalemma, nuclear membranes and other cytomembranes was highly evident. On the other hand, in heavier hydrocarbons media, vesicles, multivesicular bodies, vacuoles and groups of parallel membranes resembling smooth endoplasmic reticulum were seen. Mitochondria were not observed in both cases. Samples from heavier hydrocarbon media revealed disperse and irregularlly disposed nuclear material, associated to a high density of free ribosomes, including the presence of rough endoplasmic reticulum. Ribosomes were not observed in lighter hydrocarbons. The UV spectra of aqueous extracts of four substances fractionated by HPTLC are indicative of the presence of indole derivatives. These extracts and aqueous extracts lacking pigments showed a positive reaction to the Lowry test for protein: 0.185 mg/ml agar-agar; 0.290 mg/ml agar-mineral oil and 0.365 mg/ml agar-kerosene. SDS-polyacrylamide gel electrophoresis of two samples from a light hydrocarbon medium showed three main bands with molecular weights ca. 8,000, 40,000 and 50,000 daltons. Aqueous extracts from several hydrocarbons showed a positive hemagglutinating activity and precipitation of glycoconjugates, suggesting the presence of lectins.

Investigations about the ultrastructure of organisms developed in extreme conditions are of particular importance for the researches in cell and evolutionary biology. Comparative studies on the physiological characteristics of this organism grown in several conditions may be one of the most fruitful ways to study its adaptation capacities when the development of these characteristics has been induced mainly by several *in vitro* environmental factors during the experimental process. Hence, these investigations may determine the limits of the biological extremes. The information resulting from these studies could contribute to the problems of the origin and early evolution of life on our planet and searching for extraterrestrial life in the universe.

Dose, K. and Klein, A: 1996. *Origins of Life Evol. Biosphere* **26**: 47.
Horneck, G: 1993. *Origins of Life Evol. Biosphere* **23**: 37.

P5.30

MOLECULAR SIMULATIONS OF PROTOCELLULAR MEMBRANE FUNCTIONS

Andrew Pohorille[a,b], Michael A. Wilson[a,b], Karl Schweighofer[a,b], Christophe Chipot[c], and Michael H. New[a]

[a] Exobiology Branch, NASA Ames Research Center
[b] Department of Pharmaceutical Chemistry, University of California, San Francisco
[c] Laboratoire de Chimie Theorique, U.M.R. C.N.R.S. No 7565, Universite Henri Poincare-Nancy I

The emergence of the earliest forms of cellular life — protocells — was a central step in the evolution of simple organic matter into the present-day diversity of life. In this fundamental step, organic material assembled into membrane-bounded structures that acquired the capabilities necessary for self-maintenance, growth and replication. Several of these capabilities, such as acquisition of organics from the environment and bioenergetics, are mediated by membranes. The assumption of the continuity of evolution implies that, at some stage, membrane-mediated, protocellular functions must have been performed by peptides, which eventually evolved into the membrane-integral proteins of modern cells. This assumption, however, does not imply that peptides were the first or only functional molecules in protocells. Thus, the significance of membrane-related protein functions does not depend on a specific scenario for the origin of life.

It is well known that short, isolated peptides are typically disordered in water. Thus, to play a role in protocellular evolution, simple membrane peptides must have fulfilled three conditions. First, they must have been able to adopt an ordered structure in contact with the membrane. Second, they must have become inserted into the membrane and associated to form higher-order structures, such as transmembrane channels. Finally, such structures must have been capable of performing functions. Simple peptide models of membrane-related functions have been developed and their structure, stability and mechanism of action have been examined using computer simulations. These simulations have been performed on detailed, realistic models acting in appropriate cellular environments, so predictions based on these simulations are directly testable experimentally.

The mechanism by which peptides fold into ordered structures at the water-membrane interface was studied through the example of an 11-mer of poly-L-leucine. Initially placed as a random coil on the water side of the interface, the peptide folded into an α-helix in 36 ns. Simultaneously, the peptide translocated into the membrane-mimetic side of the interface. The folded peptide was preferentially oriented parallel to the interface but occasionally rotated to adopt a transmembrane orientation.

The aggregation of folded peptides into larger, transmembrane structures was investigated using peptides formed from hydrophobic leucine (L) and polar serine (S) with sequence $(LSLLLSL)_3$. Four such peptides, once folded into amphiphatic α-helices, can associate in the membrane to form ion channels lined by the hydrophilic serine side chains. Single helices remain adsorbed at the water-membrane interface but can adopt a transmembrane orientation in the presence of a transmembrane electric field. Once in a transmembrane orientation, the monomers associate to form dimers which, subsequently, further aggregate into tetrameric channels. An alternative mechanism, wherein dimers are first formed parallel to the interface and then insert into the membrane, was found to be unlikely.

Channels formed from tetramers of α-helices can transport protons as well as larger ions. A protobiologically relevant model of such a channel was constructed of 25 residue-long peptides based on a transmembrane fragment of the Influenza A M_2 protein. Despite its simplicity, this channel transports protons with remarkable efficiency and selectivity. The channel pore is occluded by a "gate" of four histidines located near its center. Two mechanisms of gating have been proposed. In one mechanism, all four histidines become protonated and the gate opens due to repulsion between their positive charges. In the alternative mechanism, a proton is captured on one side of a histidine in the gate while another proton is released from the opposite side. The gate returns to its initial state through tautomerization. The results of simulations of the channel embedded in a hydrated phospholipid membrane are consistent only with this latter mechanism.

In summary, the simulations demonstrate that, in the presence of membranes, simple peptides with proper sequences of hydrophobic and hydrophilic residues can adopt ordered conformations and aggregate to form functional structures. These results provide clues to the design of such structures that can be used to construct laboratory models of protocells.

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P5.31

DETECTION SYSTEM OF MICROORGANISMS AND ORGANIC COMPOUNDS ON MARS

Takeshi Saito[1], Yukishige Kawasaki[2], Atsuo Miyakawa[3], Yukihisa Funatsu[4] and Kensei Kobayashi[4]

[1] ICRR, University of Tokyo, Tanashi 188-8502, Japan
[2] Laboratory of Bioimages, Mitsubishi Kagaku Institute of Life Science, Machida, Tokyo 194-8511, Japan
[3] Hamamatsu University of Medicine, Photon Medical Research Center, Hamamatsu, 431-3192, Japan
[4] Dept. of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan

A fluorescence microscope system for detecting living microorganisms, the past microorganisms and organic compounds on Mars has been developing. Special fluorogenic dyes are used to detect microorganisms in soil samples(Kawasaki, 1996). It is shown that combinations of the dyes specific for nucleic acids, plasma membranes and enzymes discriminates microorganisms from soil backgrounds, and that the selected usage of dyes make possible to detect a artificial pre-biotic cells like protenoides and a lump of organic matter.

In order to decide whether we have to take soil samples for the fluorescent analysis above described, the reflecting light from the bottom surface of drilling holes is observed through optical fibers which are similar to the gastric camera. Test measurements of the reflecting light spectrum showed that polycyclic aromatic hydrocarbons which were found in Martian meteorite ALH84001 or aggregates of bacteria were clearly visualized.

Kawasaki, Y. and Tsuji, T.: 1996, Viva Origino **24**, 293.

P5.32

FORMATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) FROM ACETYLENE BY HEATING EXPERIMENTS: IMPLICATIONS FOR THE ORIGIN OF PAHS IN THE MARTIAN METEORITE

Akira Shimoyama, Hideki Kokubu, and Hajime Mita
Department of Chemistry, University of Tsukuba, Tsukuba 305-8571, JAPAN

PAHs were found in the Martian meteorite (ALH 84001) and reported to be relic of the past Martian organisms (McKay *et al.*, 1996). It may be possible to explain the PAHs to be abiotic in origin because of the presence of PAHs in space and meteorites. We performed heating experiments in laboratory in order to examine formation of PAHs from acetylene in the presence of $CaCO_3$ and Na-montmorillonite.

In a reaction glass tube, $CaCO_3$ or Na-montmorillonite was placed and acetylene was passed through the tube at a rate of 10 ml/min for up to 48 h at 80, 110 and 180 ℃. After the heating, the sample of $CaCO_3$ or Na-montmorillonite was extracted with hexane followed by benzene. The residue of the extracted sample was then pyrolized to 600 ℃.

The hexane extract contained benzene, alkylbenzene and some other small PAHs in size. The benzene extract showed more than 30 kinds of PAHs consisting from naphthalene to perylene and benzopyrene with many alkyl-substituted PAHs in the heating experiment with $CaCO_3$ at 180 ℃. On the other hand, the benzene extract of the Na-montmorillonite sample showed more than 50 kinds of PAHs including up to coronene and the yield of about 3 times of $CaCO_3$ at 180 ℃. The pyrolyzed $CaCO_3$ sample did not show a significant yield of PAHs, while the clay sample showed a notable yield of PAHs from benzene to pyrene.

The results of this study indicated that PAHs are rather easily formed from acetylene. The question of its presence on the Martian surface may be answered by a possible occurrence of carbide minerals, especially CaC_2.

Mckay, D.S., Gibson, E.K., Thomas-Keprta, K.L., Vali, H., Romanek, C.S., Clemett, S.J., Chillier, X.D.F., Maechling C.R., and Zare, R.N.: 1996, Science **273**, 924.

P5.34

ULTRAVIOLET RESONANCE RAMAN SPECTRAL BIOMARKERS FOR EXTRATERRESTRIAL GEOBIOLOGY

M. C. Storrie-Lombardi, A. I. Tsapin, G. D. McDonald, and K. H. Nealson, Jet Propulsion Laboratory, California Institute of Technology, MS 183-301, 4800 Oak Grove Drive, Pasadena, CA 91109

Sample return/containment strategies and *In Situ* exploration missions demand high resolution, nondestructive detection of possible biotic material in a mineral background without relying on evidence of current metabolic activity.

Laser Raman spectroscopy is a nondestructive technique producing significantly sharper spectral bands than infrared absorption and other reflectance/emission spectroscopies. Raman event efficiency increases in the ultraviolet (UV) since it is a function of the fourth power of frequency. In addition, most biological tissue is fluorescence-quiet between 200-260 nm, thus removing the major source of background noise from the Raman experiment. Excitation at 224nm and 248nm produces specific resonance enhancement for amino and nucleic acids, respectively. It has been known since 1992 that the resulting enhancement of spectral signatures by as much as 10^6 is sufficient to (1) permit detection of an average concentration of terrestrial bacteria in 15 seconds and (2) selectively identify viruses, bacteria, fungi, and spores.

We have employed ultraviolet resonance Raman (UVRR) spectroscopy to detect (1) biological signatures for aromatic amino acids, nucleic acids, proteins (cytochrome c3), and bacteria (*S. putrefaciens* and *E. coli*); (2) mineralogical signatures for diamond, calcium carbonate, kunzite, and tremolite; and (3) organic signatures of interest to planetary sciences and origin of life efforts including tholins and polyaromatic hydrocarbons (PAHs). We have demonstrated for the first time the ability to detect simultaneous biological and mineralogical UVRR signatures, specifically cyanobacteria embedded in sandstone. This organism and its environment are of particular importance in models for the survival of early Martian life during a shift from a wet to a dry/UV-rich Mars surface. Significantly we find BOTH biological and mineralogical targets are fluorescence-free in the deep UV providing exceptionally good S/N response for the UVRR event. Prior to our work this was known to be the case for biological targets, but minimal data existed for mineralogical samples.

P5.35

USING X-RAY COMPUTER TOMOGRAPHY (CT) FOR IMAGING BIOLOGICAL SYSTEMS INSIDE ROCKS.

A.I. Tsapin, M.C. Storrie-Lombardy, and K.H. Nealson
Jet Propulsion Laboratory, MS 183-301, 4800 Oak Grove Dr.,
Pasadena, CA 91109

Only 20-30 years ago the common wisdom was that life can exist under very narrow set of conditions such as temperature, pH, pressure, etc. This paradigm started to erode after discovering microorganisms capable to live at temperatures above 100° C. Probably now it's difficult or may be even impossible to find here on Earth environment (loosely called as extreme environment) without microorganisms. Microorganisms are able to adapt to almost any conditions, as soon as environment can provide source of free energy. Astrobiology is facing now challenging problem: after getting samples from Mars we need to answer the question about the possibility of life on Mars now or in any previous period of Martian history. Discoveries of microorganisms in permafrost samples several hundred thousand years old, and in deep oil fields and mines as well as endolithic bacteria require to develop a new technologies. This new technologies should allow us to detect life or remnants of life in samples from different environments, including samples from Mars, without disturbing samples. We need a non-invasive technique to use it as a first cut method to localize potentially the most promising areas of samples.

In this study we suggested to use computer tomography (CT) for that matter. We were able to image layers of algae, lichens and fungi inside and on the surface of rocks from Antarctic Dry Valley. CT data were supported with optical and electron microscopy studies. We are in process of improving this technique to achieve better spatial resolution, which now is about 300 – 500 micron.

P5.37

STROMATOLITES : FIRST MEGASCOPIC EVIDENCE OF MICROBIAL COMMUNITIES ON EARTH AND SEARCH FOR MARTIAN MICROBIAL BUILDUPS

VINOD C. TEWARI
Wadia Institute of Himalayan Geology, Dehra Dun- 248001, India

Stromatolites or Microbialites are Microbial buildups of laminated carbonate rocks formed by benthic microbial communities. These structures are well preserved in Archean sediments and confirm the first megascopic evidence of life on Earth. Fossilised bacteria is also reported from stromatolitic structures.

A comparative study of early Earth and Mars with special reference to the origin and early evolution of life on Earth suggest that similar conditions would have been initially present on Martian surface (Davis and McKay, 1996). The reports of filamentous bacteria and poly cyclic aromatic hydrocarbons from Martian meteorite ALH 84001 (McKay et al, 1996) is an important clue for possible presence of microorganisms on Mars. An extensive search of Martian surface and subsurface for stromatolites and other megascopic fossils like Vendobionts would definitely locate sites of early life on Mars. (Tewari, 1997, 1998).

Stromatolites and other traces of biological origin may be detected in future by Mars rover well equipped with sophisticated photographic and chemical equipments (Knoll, 1998). The first record of stromatolites from Mars will begin a new chapter in extraterrestrial palaeontology or palaeoexobiology.

Davis, W.L. and Mc Kay, C. : 1996, Origins of Life and Evolution of the Biosphere, **26**, 61-73.

Knoll, A.H.: 1998 , The Sciences, 20-26.

Mc Kay, D.S., Gibson, E.K. et al. : 1996, Science, 273, 924-930.

Tewari, V.C.: 1997, *Exobiology*, Kluwer Academic Publishers, The Netherlands, 261-265.

Tewari, V.C. : 1998, Role of Solar Energy in Origin of Life and Early Evolution (Abst.), 3,

P5.38

DEVELOPMENT OF SENSITIVE ANALYTICAL METHODS FOR THE DETERMINATION OF AMINO ACIDS AND THEIR ENANTIOMERIC RATIO IN MICROMETEORITES (CHLOROETHYLNITROSOUREA POTENTIALITIES AS NEW DERIVATIZING REAGENT FOR EC-LIF AND ELISA.)

O. Trambouze-Vandenabeele[1], M-F. Grenier-Loustalot[1], D. Despois[2], M. Dobrijevic[2], A. Commeyras[3], G. Geffard[4], C. Engrand[5], M. Perreau[6], F. Robert[7].

1/ Service Central d'Analyse - CNRS, BP 22, Echangeur de Solaize, 69390 VERNAISON, 2/ Observatoire de Bordeaux-France, 3/ UPRES-A 5073 Montpellier-France, 4/ INSERM, Bordeaux-France, 5/ CSNSM, Orsay-France, 6/ Université Paris VII -France, 7/ Muséum d'Histoire Naturelle, Paris-France.

Micrometeorites (typically 200 mu in diameter) represent the major fraction of the extraterrestrial input to Earth. They are carbon rich and close to the primitive material of the solar nebula. They are believed to be decelerated softly during their entry into the atmosphere, which lowers the risks of alteration and racemization of the organic matter they contain. Two important difficulties of the measurement of the abundance and enantiomeric ratio of amino acids (AA) in micrometeorites are the small quantity of compounds, and the risks of contamination during the collection and analysis process. The analytical methods have thus to be adapted to these constraints and should allow to quantify reliably less than10^{-15} moles of amino acids. The methods most commonly used up to now for this purpose are high performance liquid chromatography (HPLC-fluorescence, with OPA derivatization) or gas chromatography with mass spectrometer detector (GC-MS, with double derivatization: esterification and acetylation). The former was used recently by Brinton et al. (1998) to measure the AIB concentration and an upper limit on isovaline in samples of ~30 micrometeorites. These sensitive methods (limit of detection 10^{-15} for OPA, limit of quantification at least 3 times higher) do not allow an easy protection against contamination which is a crucial point if biological amino acids like alanine are to be analyzed.

In this frame, our research rely on a new derivatization method based on chloroethylnitrosoureas (CENU) which was first developed and used for the measurement of small amounts of organic polluants (pesticides in water, amines in food). As the derivatized compounds obtained with CENUs are relatively stable (formation of urea) it is possible to form them very early in the analytical process, and thus to reduce the number of steps where contamination can happen. Furthermore the structure of CENU (chromophore part) can be tailored to match the analytical needs. The introduction of an asymmetry center allows the formation of diastereoisomers, and thus the measurement of the enantiomeric ratio on non-chiral columns. Two types of CENUs have been developed, one for capillary electrophoresis with laser induced fluorescence, the other one for immunoenzymatic assays.

P5.38 continued

These methods have (to our knowledge) never been used in this context. In addition, more classical approaches like GC-MS and MS-MS are under test to serve as "reference", both with respect to the new methods and to the results already obtained with these classical methods. The MS-MS method is interesting to perform a rapid estimation of the AA content, but does not give access to the identification of each AA and to the enantiomeric ratio.

i6.1

THE HABITABILITY OF EARLY MARS

Michael H. Carr

U. S. Geological Survey, Menlo Park, CA 94025

The channels and valleys incised into the martian surface provide persuasive evidence that water has played a prominent role in the evolution of Mars. Geomorphic evidence of warm surface conditions, an extensive groundwater system, and voluminous volcanism all suggest that early Mars had numerous habitable niches. The large flood channels appear to have formed episodically throughout Mars' history, probably by catastrophic eruptions of groundwater or, less commonly, by drainage of large lakes. Their formation may have required climatic conditions similar to those that prevail today. In contrast, branching valley networks indicate warm conditions in the past. Whether they formed mainly by precipitation and surface runoff, or mainly by seepage of groundwater, warmer conditions are needed to enable precipitation, to recharge the groundwater system, and to permit continuous flow in modest-sized streams. Since most of the valley networks are in terrains that formed at the end of heavy bombardment and shortly thereafter, the valley networks are taken as evidence for a warm and wet early Mars. This conclusion is supported by a dramatic change in erosion rates at the end of heavy bombardment.

Warm surface conditions are difficult to achieve on early Mars because of its distance from the Sun and the low output of the early Sun. The problem of warming early Mars is further aggravated by erosion of the atmosphere by large impacts and by removal of CO2 by weathering when temperatures rise. An additional, controversial issue is the effectiveness of greenhouse warming on early Mars. A possible solution to the vulnerability of an early CO2 atmosphere to collapse is that CO2 removed from the atmosphere by weathering is slowly recycled back into the atmosphere by volcanic burial. While in the ground the CO2 (as carbonates) is protected from removal by impact until impact rates have significantly declined.

i6.2

MARS VOLATILES AND CLIMATE SURVEYOR (MVACS) INTEGRATED PAYLOAD FOR THE MARS POLAR LANDER MISSION

D. A. Paige (UCLA), W. V. Boynton (UA), D. Crisp (JPL), E. DeJong (JPL), A. M. Harri (FMI), C. J. Hansen (JPL), H. U. Keller (MPAe), L. A. Leshin (ASU), R. May (JPL), P. H. Smith (UA), R. W. Zurek (JPL)

The Mars Volatiles and Climate Surveyor (MVACS) integrated payload for the Mars Polar Lander will be launched in January, 1999, and land on Mars' south polar layered deposits in December, 1999. Over the course of its 90-day nominal mission during the Martian southern spring and summer seasons, it will make in-situ measurements which will provide new insights into the behavior and distribution of Martian volatiles. MVACS consists of four major instrument systems: A Surface Stereo Imager (SSI) which will acquire multi-spectral stereo images of the surface and atmosphere; a 2-meter Robotic Arm (RA) which will dig a 0.5 meter deep trench and acquire surface and subsurface samples which will be imaged by a focusable Robotic Arm Camera (RAC) which will take close-up images of surface and subsurface samples at a spatial resolution of 21 microns; a Meteorology Package (MET) which will make the first measurements of surface pressure, temperature and winds in Mars' southern hemisphere and employ a Tunable Diode Laser (TDL) spectrometer to measure the water vapor concentration and isotopic composition of carbon dioxide in the Martian atmosphere; and a Thermal and Evolved Gas Analyzer (TEGA) which will use differential scanning calorimetry and TDL evolved gas analysis to determine the concentrations of ices, adsorbed volatiles and volatile-bearing minerals in surface and sub-surface soil samples. The unique in-situ measurements made by MVACS at its high-latitude landing site will define a number of important aspects of the physical, isotopic and chemical nature of the Martian near-surface and sub-surface environment which will be valuable for better understanding of Mars meteorites and returned samples, as well as the search for Martian resources which could be utilized by humans.

c6.3

EVIDENCE FOR MINERALIZED BACTERIA IN THE MARTIAN METEORITE NAKHLA

David S. McKay[1], Susan J. Wentworth[2], Frances Westall[3], Kathie L. Thomas-Keprta[2], and Everett K. Gibson, Jr.[1]
[1]SN, NASA Johnson Space Center, Houston, TX, 77058; [2]Lockheed Martin, 2400 NASA Rd. 1, Houston, TX, 77058; [3]NRC, NASA Johnson Space Center, Houston, TX, 77058.

Nakhla, a meteorite which fell in Egypt in 1911, is one of the 13 known martian meteorites. Like the others, it is an igneous rock. Gooding et al. (1991) clearly demonstrated that the trace amounts of alteration products and secondary minerals present in Nakhla are the result of low-temperature, aqueous weathering processes on Mars. Secondary phases in Nakhla include NaCl, Ca-sulfate, Ca-carbonate, Fe-carbonate, and clay. The clay is the most abundant, and has been variously identified as iddingsite, smectite, amorphous, or a combination of all three (e.g., Gooding et al., 1991). Because it is known that these secondary minerals are martian, and because the meteorite has had negligible exposure to the terrestrial weathering environment, Nakhla provides an excellent opportunity to search for possible martian biogenic material.

Using optical microscopy and field emission scanning electron microscopy (FE-SEM), we find scattered occurrences of round to ovoid objects in chips of Nakhla; individual objects range from ~0.2-1 micrometers in diameter. Some of them are found on surfaces formed by shock fracturing that occurred on Mars, but some are embedded within fine-grained martian clay veins. FE-SEM studies show strong evidence that the latter type is embedded in the clay, and this result is confirmed by optical microscopy of thin sections (i.e., according to standard techniques used by paleontologists for identifying *in situ* microfossils; eg., Schopf and Packer, 1987). Preliminary energy dispersive X-ray spectrometry (EDS) indicates that the ovoids have compositions much like the clay matrix, with a possible Fe enrichment in some cases; these compositions are consistent with those of some terrestrial fossilized bacteria.

We suggest that the round and ovoid features in Nakhla are possibly mineralized bacteria for the following reasons: (1) uniform size: although total size range is ~0.2-1 micrometer, most are 0.5-1; (2) attachment: many are attached to each other in groups of two or more, and they are

commonly connected together in configurations identical to those of dividing bacteria; (3) surface texture: well-exposed individuals have surface textures much like those of some mineralized terrestrial bacteria; they have wrinkled surfaces typical of fossilized bacteria which have undergone osmotic stress during dehydration (Westall, 1999); (4) appendages: filamentous material attached to some individuals have a similar appearance to appendages commonly found on terrestrial bacteria. (5) mineralized biofilms(?): webby or lacy material associated with the ovoids is much like mineralized terrestrial biofilm; (6) colonies(?): ovoids and spheres are found in clusters, which are distributed sporadically in the rock; other clay mineral regions are completely devoid of these forms.

Supposing that these features are indeed fossilized bacterial remains, then their planet of origin still has to be determined. Considering Nakhla's terrestrial history, it is hard to imagine that terrestrial bacteria would have had either the environment or the time that would be sufficient to invade, colonize, and fossilize. That possibility must be studied, however, especially in view of the fact that bacterial fossilization is known to occur on a very short timescale (i.e., weeks). Another possibility is that both terrestrial and martian biological material are present in Nakhla; if so, perhaps it will be possible to distinguish them, just as martian and terrestrial weathering effects can be distinguished in some martian meteorites.

Gooding, J.L., Wentworth, S. J., and Zolensky, M.E.: 1991, Meteoritics **26**, 135-143.
Schopf, J.W. and Packer B.M.:1987, Science **237**, 70-72.
Westall, F.: 1999, J. Geophys. Res. Planets, in press.

c6.4

AMINO ACIDS IN MARTIAN METEORITE NAKHLA

Daniel P. Glavin and Jeffrey L. Bada
Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA, USA 92093-0212

Karen L. F. Brinton and Gene D. McDonald
NASA Jet Propulsion Laboratory, Pasadena, CA 91109

Since the report that Martian meteorite ALH84001 contained possible evidence for past life on Mars (McKay et al., 1996), considerable attention has been given to the study of Martian meteorites collected in the Antarctic. Until recently little attention has been directed to Nakhla, a member of the SNC (Shergotty, Nakhla, Chassigny) class of meteorites, and the first Martian meteorite found to contain carbonates along with hydrous minerals associated with aqueous alteration processes on Mars (Gooding et al., 1991). Nakhla was an observed fall that landed near Alexandria, Egypt in an agricultural region of the Nile River Delta in 1911 (Prior 1912). One of the stones was acquired by the British Natural History Museum in 1913 and recently made available to researchers. We report here the amino acid analyses of an interior (2-3 cm from fusion crust) piece of this meteorite stone, and find that Nakhla contains a unique set of amino acids, distinctly different from the Antarctic Martian meteorite ALH84001.

A suite of protein and non-protein amino acids including aspartic and glutamic acids, glycine, alanine, β-alanine and γ-amino-n-butyric acid (GABA) were detected in the Nakhla bulk rock at part per billion levels (20 to 330 ppb) using high performance liquid chromatography. The extraterrestrial amino acids α-aminoisobutyric acid (AIB) and isovaline, which are among the most abundant amino acids detected in the carbonaceous chondrite Murchison (Kvenvolden et al. 1970), were not detected in Nakhla above blank levels (<1 ppb). One of the most interesting aspects of the Nakhla analyses is the presence of significant amounts of the D-enantiomers of aspartic acid, glutamic acid and possibly alanine (D/L ratios ranged from < 0.1 to 0.5). The distribution of amino acids in Nakhla as well as their enantiomeric abundance, is very similar to what we found in a Nile Delta sediment core sample collected off the

c6.4 continued

coast of Egypt, close to where Nakhla fell (Einsele and Werner, 1968). Many of the Nakhla stones suffered heating and cracking during atmospheric entry and were found buried to depths of 10 to 30 cm in the Nile Delta sediment. Thus, it is possible that bacterially derived amino acids in the sediment porewater penetrated into the interior of the meteorite shortly after its fall. Although our analyses strongly suggests that the amino acids in Nakhla are terrestrial in origin, we can not completely rule out that some of the amino acids in Nakhla were present in the meteorite when it fell to Earth. The contamination of Martian meteorites is evidently a rapid process which unfortunately greatly compromises the possibility that these meteorites can be used to access whether organic compounds important in biology as we know it are present on Mars.

Einsele, G. and Werner, F.: 1968, *Forschungsergebnisse. Reihe. C.,* No. 1, p. 21.
Gooding, J. L., Wentworth, S. J. and Zolensky, M. E.: 1991, Meteoritics **26**, 135.
Kvenvolden, K. A. et al.: 1970, Nature **228**, 923.
McKay, D. S. et al.: 1996, Science **273**, 924.
Prior, G. T.: 1912, Min. Magazine **16**, 274.