Viva Origino

VOL. 24 (No. 4)

December 1996

Special Issue:

"Search for Life on Mars"

The Society for the Study of the Origin
and Evolution of Life
JAPAN

生命の起原および進化学会 会則

地球上における生命の起原を科学的に解明することと，生物進化の攻究により，生命体の本質を明らかにしようとする。本学会は，関係諸分野の英知を集め，互の連繋によって新しい型の総合科学を確立・発展させることにより，上記の目的達成を期するものである。

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Viva Origino

VOL. 24 (No. 4)

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The Society for the Study of the Origin
and Evolution of Life
JAPAN

特集〝火星の生命探査〞

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「火星の生命探査」の特集にあたって

Preface to the Special Issue on "Search for Life on Mars"

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1996年8月、NASAのD. McKayらは、火星から飛来し、南極で回収された隕石 ALH84001を分析し、火星上の生命の痕跡を検出したと発表した。この発表を機に、火星の生命探査に関する議論が活発になされるようになった。

この特集「火星の生命探査」は、1995年3月に松山で開催された本学会、第20回学術講演会において行われたミニシンポジウムを中心に組まれたものであり、D. McKayらの報告以前にわが国で火星の生命探査の重要性を議論したという点で画期的なものであった。斉藤威（東大宇宙線研）の解説においては、国際協力による火星生命探査"Return-to-Mars-Together"計画について紹介している。小林憲正（横浜国大）、石川洋二（大林組）、C. McKay (NASA Ames) は現在までの火星に関する知識をもとに、火星上での有機物や生命の存在の可能性について議論している。また、河崎行繁（三菱生命研）は火星上の微生物の検出法について新しい提案を行っている。さらにChernavskii ら(Lebedev 物理研)は生物学的情報の起源について議論を行っている。

本特集が今後の火星探査の議論のたたき台となることを期待する。

火星生命探査計画
Return-to-Mars-Together

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要旨

原始惑星大気の化学組成や彗星氷の組成を模した標的に、宇宙放射線を模した加速器ビームや紫外線などを照射すると、アミノ酸などの生体有機分子が効率良く生成される。これら生体有機分子の生成は宇宙の普遍的現象と言える。事実、星間雲や隕石、彗星ガスの中に検出されている。このような普遍的物質が、宇宙の中で火星にだけ存在しないということは、極めて不自然である。この観点から、1970 年代のバイキング計画の結果は、酸化土壌には生体有機分子は存在しないということを、最近の南極隕石 ALH84001 の結果は、火星にも生体有機分子が存在するという、極めて当然のことを証明したに過ぎない。生体有分子や水の存在は、生命が存在するための一つの必要条件に過ぎない。生命の死骸はこれらの有機分子を作るが、逆は真ならず、生体有機分子の存在は生命存在の如何なる証明にもならない。では火星の生命の存在は如何なる方法で検出できるのだろうか。火星生命を検出する方法論が検討され、現在進行中の火星生命探査のための国際共同計画, Return-to-Mars-Together project が報告される。

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SEARCH FOR LIFE ON MARS

Return-to-Mars-Together

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(Received December 9, 1996, Accepted December 16, 1996)

ABSTRACT

The Return-To-Mars-Together project has been organized as an international cooperative work to search for bioorganic compounds and microorganisms which might be found in underground of the places where the traces of water were found or near the poles of Mars. Strategies to certify the existence of life on Mars are presented. Four instrumental methods and the best places for landing on Mars are under discussion.

KEY WORDS: Mars, Bioorganic Compounds, Organisms

1. INTRODUCTION

Is there life on Mars? Had life ever been on Mars? What is the best way to confirm the existence of life on Mars? Recently, McKay et al. insisted that they found evidence of primitive life in the

meteorite from Mars called ALH84001 that found in 1984 in Allan Hills ice field, Antarctica[1]. They concluded that a number of the experimental phenomena, if they are considered collectively, show the evidence for primitive life on early Mars. However it is extremely difficult to accept their conclusion, even if their observations themselves are reasonable. The dead materials of life will turn into occasionally polycyclic aromatic hydrocarbons (PAHs). However, converses are not always true. That is, the PAH concentration, even in the interior of meteorites, never confirm the existence of life. The existence of water and bioorganic compounds is one of necessary condition, a *sine qua non*, for the existence of life. It is commonly known that life is never created in laboratory, even if a plenty of water and bioorganic compounds is given. As authors mentioned, none of their observations is in itself conclusive for the existence of past life. From a logical point of view, if an argument does not contain "the partial truth", a million collections of such an argument never reaches the truth. As discussed in section 3-1, bioorganic compounds like amino acids are easily formed artificially as well as naturally. In fact such compounds have been found in interstellar clouds and meteorites. The PAHs observed by McKay [1] are also popular chemical molecules that have been found in space. It is extremely difficult to invent a theory that bioorganic compounds do not exist only on Mars in Universe. In this sense, the Viking negative results in the 1970's only mean that there are neither bioorganic compounds nor organisms in strongly oxidizing soils of Mars. McKay's report only certifies natural results that there are organic compounds in normal Martian soils. Thus we are standing at a starting point to search for life on Mars. Is there life on Mars?

What kinds of phenomena can really demonstrate the existence of the past and present life on Mars? It depends on the philosophical consideration on life; What is life? How is life? The strategies for detecting life and Return-to-Mars-Together, an international program to search for organic compounds and organisms on Mars, are discussed in this paper.

2. RETURN-TO-MARS-TOGETHER

A proposal to search for life on Mars was presented first at a meeting that was organized specially at Babakin Space Center, Moscow in April of 1994. At that time, there were no plans to search for life on Mars in the world, because the negative results of Viking mission have been discouraging to propose such a project. It is well known that Mars and Earth were much alike at the beginning phase of their geological histories. Observations of old riverbeds and mud channels suggest that a substantial amount of water once flowed Mars. These indicate the possibility that a future mission will find evidence of life on Mars, either still livings like bacteria, or cellular-structure-materials in fossilized form which might be frozen for 3.5 billion years in underground of Mars, like dense microbial mats in the bottom of perennially frozen lakes in Antarctic. It is most important for life search to get samples without oxidizing. After our proposal titled by "Possible Existence of Bioorganic Compounds and Life on Mars and their detection", Babakin center had investigated technical possibilities of our proposal, and decided that our instrument to search for bioorganic compounds and living organisms must be mounted in their nearest Mars mission. Thus, Bio-Mars project to search for life on Mars was started first as Japanese-Russian collaboration in April, 1994 [2]. Babakin Center started to explore the techniques to choose the best landing place for life search from orbit of Mars satellite and to take soil samples from a depth up to several meters at various places of Mars.

The RETURN-TO-MARS-TOGETER was named by the late C.Ponnamperuma at the conference on the structure and model of the first cell, the Alexander Ivanovich Oparin 100th. Anniversary Conference, which was held at Trieste in September 1994. He had been working hardly to bring up our program to an international project including US and EC, but he went to the better world in December 1994. A general consideration for RETURN-TO-MARS-TOGETER project is shown in Table 1, which was agreed at the first group meeting in the conference on Physics of The Origin and Evolution of Life, Cyril Ponnamperuma Memorial, which was held at Trieste in September 1995.

Table 1. An Agreement of RETURN-TO-MARS-TOGETHER Group

GENERAL PRINCIPLES
For
RETURN-TO-MARS-TOGETHER Project

International cooperative works to search for bioorganic compounds and organisms on Mars

1. Develop the best instrument to search for bioorganic chemical compounds and organisms on Mars, and operate the instrument on Mars as early as possible, within this century. Prepare the instrument to be available for the earliest mission from any nation. It is assumed at present that Russian Mars-98 will be the nearest mission for bioorganic search on Mars.

2. The proposed instrument to be studied consists of the four kinds of probes;
(1) Mass spectrometer to measure bioorganic chemical compounds,
(2) Fluorescent microscope to directly image organisms,
(3) Incubating method to detect activities of organisms,
(4) Polarimeter in combination with chiral chromatography to look for homochirality.

We have begun a local collaboration to study the best method in each probe. Test instrument prototype in Mars-like environments on Earth, particularly the dray valleys of Antarctica.

3. Determine the site on Mars for the landing and the best samples to be taken. Sample soils must be taken under ground in old (a few billion years) geologic formation.

4. Organization

Carry out the program as an international cooperative project by scientists and technicians throughout the world who are interested in, or already working in this field.

3 DETECTION STRATEGIES FOR LIFE ON MARS

Four instruments were proposed to be studied as Return-to-Mars-Together group, as shown in Table 1. We are forced to prior to few instruments out of a number of proposals because of the weight limitation, for example 10-20 Kg totally in case of the nearest Russian Mars mission which is expected in 2001. We have to select the best method that can really demonstrate the existence of life within the weight limitation. The first two (1) and (2) in Table 1 were proposed from Japanese side [3, 4]. The third (3), an incubating method, which was proposed by USA side, is the same in principle as Viking policy. Because the existence of life must be confirmed by detecting an activity of living life, that is the change of out gases from metabolism. A small polarimeter (4) to detect homochirality in organic compounds was proposed by A.MacDermott [5]. Table 2 shows the detection strategies to search for life on Mars.

Table 2. Targets for Life Search on Mars

OBJECTS		METABOLISM	
Chemicals	Structure	Enzymes	Out Gases
Amino acids	Shape/Form	Hydrolase	CO_2
Nuclear acids	Cells	Isomerase	CH_4
Sugar	Membrane	Ligase	SO_2
Lumps of Compounds	Motions	etc.	etc.
Chirality			

3-1 Bioorganic Compounds and Their Chirality

From our accelerator experiments simulating interactions of cosmic rays with atmosphere in primitive Earth and other planets, and with cometary ice, we found that the bioorganic compounds like amino acids are easily formed in a simple chemical mixture by bombarding charged particles [6-8]. As the starting gas mixtures, any kinds of proper carbon compounds (carbon monoxide or methane) and nitrogen compounds (nitrogen or ammonia) are acceptable. It is well known that the elements of H,C,N and O are the highest 4 in abundance except stable He nuclei in our Galaxy as well as in other

galaxies. On the other hand, cosmic rays that are one of the most effective energy source [9] for chemical evolution are distributed uniformly in Galaxy. These facts indicate that bioorganic compounds are forming from popular molecules by universal cosmic energy everywhere in Universe. In fact the precursors of amino acids have been found in cosmic clouds, cometary atmosphere and meteorites. Bioorganic compounds as amino acids must be found in Martian soils, if they were not oxidizing samples that were taken from underground. Mass spectrometry to measure chemical compounds was discussed by Kobayashi et al.[3]. Although bioorganic compounds are the fundamental targets to be detected on Mars, it never confirms the existence of life, because the existence of bioorganic compounds is a necessary condition for the existence of life. Even if, a small ellipsoidal lump made of amino acids was found in Martian soils, such phenomenon never certifies that the lump is the remains of the past life.

One of the most important signals of life might be its homochirality because all biological molecules have one side chirality. We have to measure homochirality in order to conclude that the lumps of compounds were turned from the remain of the past life. What is the origin of homochirality? If the homochirality is attributed to universal reason, such as effects by the polarized radiations from neutron star or by the weak interactions, Martian compounds must be our hand, that is L-amino acids and D-sugar. If it was formed by chance, we shall find other hand compounds in Martian soils with 50 % probabilities.

3-2 Out Gases of Metabolism

Viking policy in search for life is to detect an activity of life, that is, to detect the change of out gases from metabolism process. If the negative results of the Viking mission are attributable to the strongly oxidizing soil samples, this method must be still effectual to detect life on Mars. The USA group plans to construct the modern instrument under the Viking policy for future mission. However, it is uncertain whether the nutriments such as glycine and formic acid used in Viking fitted to organisms in the Martian soils. It is possible that Martian organisms were living underground and they

have found a different mechanism of energy metabolism from photosynthesis, and that Martian organisms had not enough time to evolve up to phototroph. If it is the case, the incubating under the light used in Viking should be not reasonable way to detect life. Another serious problem is that out gases from inorganic chemical reactions with the soil materials might be more abundant than those of metabolism, even if organisms in the soil samples are producing the gases such as CO_2 and CH_4.

3-3 Detection of Enzymes

Another way to detect an activity of life is to detect enzymes in organisms, because all the chemical reactions in organisms should be the enzyme reactions. What kinds of enzyme reactions, transferase, hydrolase, isomerase or ligase etc., are the best to confirm the existence of life. Kawasaki has proposed to detect estraze in cells by taking a fluorescent microscopic picture with stain of SFDA (sulfofluorescein diacetate) [4]. The SFDA, which is not fluorescent, penetrates easily into cytoplasm because it is hydrophobic and permeable. When SFDAs are decomposed by estraze in the cells, they become fluorescent and hydrophilic in the cells. Thus, SFDA is accumulating in the cells because they are now hydrophilic and cannot penetrate cytoplasmic membranes. The fluorescence with SFDAs means the evidence of vital cells, that is microorganism, because esterase exists universally in the cells except virus and bacteriophage. It is required to study another stains to identify other enzyme reactions.

Another stain, ANS (8-anilinonaphtalene 1 sulfonate) has been proposed by Kawasaki [4]. The ANS generates fluorescent light when it is absorbed in membranes of the cells. The cell, if it is larger than 1 μ m in diameter, will be seen as a fluorescent ring. The ANS images indicate not only vital membranes but also dead cells which might be found in fossilized form on Mars. However ANS images not always separate the remains of life from organic compounds in bulk. The advantage of this method is to be able to detect organisms even if it is a single cell. The disadvantage of this method is to requests the delicate handling, especially in the procedures of wet chemistry at the heavy environment condition on Mars.

3-4 Motions of Livings

Our consideration for life detection discussed above may be too much affected by the negative results of Viking. If we accept naturally the universal formation of bioorganic compounds and life occurrence under the serious environmental conditions on primitive Earth, we can naturally expect to find livings with a simple microscope without wet chemistry. Babakin Center plans to send an automaton called Mole on Mars in the next Mars program. The Mole can move in wide region at the depth of several tens of meter. If the Mole has microscopic eyes, she may find livings on Mars.

4 INSTRUMENT AND OBSERVATION

The original proposal for instrument in Japanese-Russian collaboration phase in 1994 is shown in Fig.1 and Table 4 as basis for designing the up-to-date instrument. Fig.1 shows a schematic diagram of the instrument. The soil samples are taken from more than 50 cm under Martian surface by boring in order to get samples

Table 4. Characteristics of Detectors Proposed from Japanese Side

	Mass Spectrometer	Fluorescent Microscope
Detection Objects	Chemical Compounds in A=1- 300 Carboxylic acids Amino acids Nucleic acid bases (Homochirality)	Microorganisms Enzymes Cell Membrane Compounds in bulk Remains of life
Sensitivity	10 p mol/ specimen 1 speci.= 30 mg	1 bacteria/field 1 speci.= 50 mg
Sampling Number At one point	6 speci.	10 speci.
Analytical time	30 min/speci.	1 min/speci.
Data Out-Put	16 bits × clock/speci.	CCD Images; 512 × 512 × 1 bytes/image 2 images/field 5 fields/speci.
Dimension	10 cm ϕ × 20 cm	30cm × 20cm × 15cm

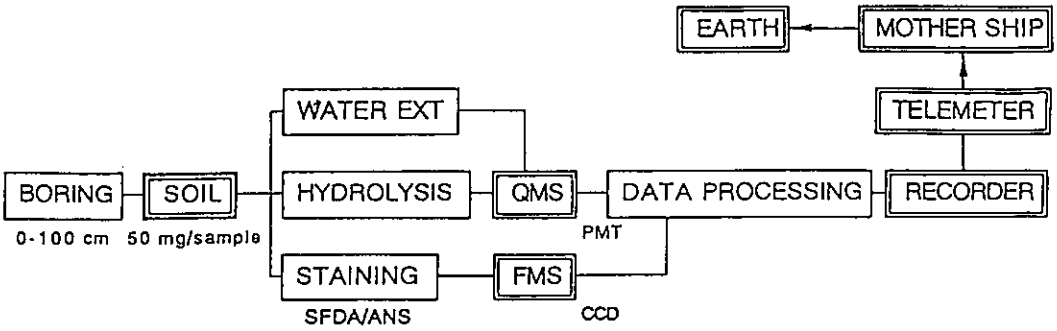

Fig. 1 A Schematic Diagram of Total System

Table 5. Weight Sharing of Each Part except boring system, power supply and data telemetry (10 June, 1994)

Sample Divider	1,200	g
Water Abstraction	400	
Hydrolysis	400	
Staining	800	
Vacuum Pump	1,000	
Microscope	2,500	
Mass Spectrometer	2,000	
Controlling System	1,000	
Data Processing	300	
Data Recording	500	
Support Structure	2,500	
Total	11,600	g

without oxidizing. The instrument consists of two kinds of detectors, a mass spectrometer to detect bioorganic compounds[3], and a fluorescent microscope (FM) to detect microorganisms on Mars[4]. The FM system consists of sample containers, a stain solution container, a fluorescence microscope with automatic focusing, and CCD camera. These techniques themselves are completely established as laboratory usage. However we need more works to achieve a compact system within the limitations of total weight and electric power and data rates in the Mars program. Table 5 shows the weight sharing for each part of the system in the Mars program.

The space competition based on national interests is not useful from scientific and economic points of view. Organization like the

international space station program is required to realize the Mars program. Return-to-Mars-Together proposes to study the best way to search for life on Mars as an international cooperative work with scientists and technicians in the world, who are interested or already working in this field.

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Appendix

RETURN TO MARS TOGETHER、Membership (8 Nov., 1995)

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火星の生命：現在、過去、そして未来

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要旨

バイキング計画の結果によれば、たぶん現在液体の水が存在しないという理由で、火星には生命が存在しないということになっている。しかしながら、火星ではかつて、とりわけ地球上で生命が最初に発生した３５億年前の頃には液体の水が存在したという証拠がたくさんある。従って、火星の生命の問題の解明は将来の宇宙探査ミッションの重要な目的となる。火星表面のかなりの部分が３５億年より古い地層なので、火星は地球よりも生命の発生に至った経緯をよく記録にとどめている可能性がある。将来には、火星がかつて謳歌した生命の住み易い環境に火星を戻すことが可能になるかもしれない。

Viva Origino 24(1996)257－266

LIFE ON MARS: PRESENT, PAST, AND FUTURE

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(Received January 24, 1996, Accepted November 5, 1996)

Abstract

The Viking results imply that Mars is devoid of life, probably due to the absence of liquid water at the present time. However, there is considerable evidence that Mars had liquid water on its surface in the past and, in particular, during the time about 3.5 Gyr ago, that life first arose on Earth. Thus, the question of life on Mars is an important goal for future missions. Because a large fraction of the martian surface is older than 3.5 Gyr, Mars may actually retain a better record of the events leading to the origin of life than does the earth. In the future it may be possible to restore Mars to the habitable state it once enjoyed.

Key Words: Mars, Viking mission, life, Antarctica, paleolake, dry valley, terraforming

INTRODUCTION

Of all the other planets Mars is the one that is the most likely to have, or to have had, life. For this reason the Viking missions to Mars had as their primary objective the search for microbial life in the sands of Mars [1]. Each of the two Viking landers carried four experiments relevant to the search for life. These were the three Biology experiments and the Gas Chromatograph Mass Spectrometer (GC/MS). The Biology experiments consisted of the Pyrolytic Release Experiment(PR), which searched for photosynthesis, the Gas Exchange Experiment (GEx) and the Labeled Release Experiment (LR), both of which sought to detect the heterotrophic consumption of organic material. Soil samples were collected from the martian surface to a depth of a few centimeters and placed into the experimental chambers. The most interesting results were obtained from the GEx and LR results. When water vapor was added in the GEx experiment there was a rapid release of oxygen [2]. In the LR experiment there was clear indication of the oxidation of organic material to form carbon dioxide [3]. Taken alone these results --- the LR results in particular --- would be consistent with a life detection.

However, the results of the GC/MS argued against the presence of life. This instrument failed to detect organic materials at ppb levels. It seems unlikely that life could exist without generating copious organic byproducts in the soil. Thus, the prevailing explanation for the reactivity seen in the Viking results is that there is present in the martian soil one or more photochemically produced oxidants [4]. An example of a photochemically produced oxidant is hydrogen peroxide and this compound is thought to be produced on Mars and has been suggested as a explanation for the LR results [5].

If there are indeed oxidants in the martian surface then they have apparently destroyed any organic material there. A key question therefore is the depth to which this oxidation processes has operated. Organic analysis would only be possible on samples collected from beneath this depth. Bullock et al. [5] have developed a coupled soil atmosphere model of hydrogen peroxide in the martian surface. They find that the depth of penetration into the soil is limited both by the catalytic decomposition of hydrogen peroxide and by non-linear absorption processes. Thus, they conclude that the depth of oxidizing conditions extends only of order meters below the surface. This would seem to be a promising result in terms of obtaining organic rich samples for analysis on future missions.

However it is important to note that the nature of the martian oxidant is not well understood. No current models fully explain the Viking data [4]. Further investigation of the nature, reactivity, and depth of oxidant on Mars is needed before a plan for organic analysis can be developed. Fortunately, instruments are being developed that will address this need [6].

Perhaps, the most interesting biological results of the Viking missions and the previous Mariner 9 mission were not from the biology experiments but from the orbital images [7]. These images clearly show the presence of features carved on the martian surface by liquid water. Many of these features represent large catastrophic outflows of water, apparently generated by the bursting of subsurface ice-damned reservoirs. The rapid flow that results from these events can be sustained even in the present low pressure, low temperature climate since the flow could continue under a relatively thin layer of ice.

However, other fluvial features seen on Mars indicate a much slower, gentler flow. These valley networks, as they are called, indicate that the surface conditions on Mars were more conducive to the presence of liquid water at one time. This, in turn, implies that Mars has a different climate with a thicker atmosphere and warmer surface temperatures. How much thicker and how much warmer the atmosphere must have been to accommodate liquid water is uncertain. However, from a biological perspective it is the presence of liquid water per se that is of interest. The nature of the atmosphere that allowed for liquid water habitats is of secondary importance. A well developed valley system on the ancient cratered terrain is shown in Figure 1.

SEARCHING FOR PAST LIFE

The fluvial features on Mars are the direct evidence that it had liquid water in the past. In the southern hemisphere of Mars there are extensive surface units that are heavily cratered indicating that they date back to the heavy bombardment 3.8 Gyr ago. Many of the valley networks are found in this terrain and interlace with the craters. This suggests that the initial period of fluvial feature formation coincided with the end of the late bombardment. Fluvial formation probably occurred before this time as well but the record of these events has been destroyed by the resurfacing associated with the heavy bombardment. There is some evidence that there were repeated instances of fluvial flow on Mars after

3.8 Gyr ago but these must have been limited in duration and extent, as indicated by the low erosion rates since that time.

As the heavy bombardment ceased, the conditions on Mars that were sustaining liquid water habitats apparently ceased as well. The carbon dioxide thought to be the main constituent of the thick early atmosphere would be rapidly lost as a result of the formation of carbonate [9]. Models of the decay of the early atmosphere suggest that liquid water habitats could have existed for about 500 million years after

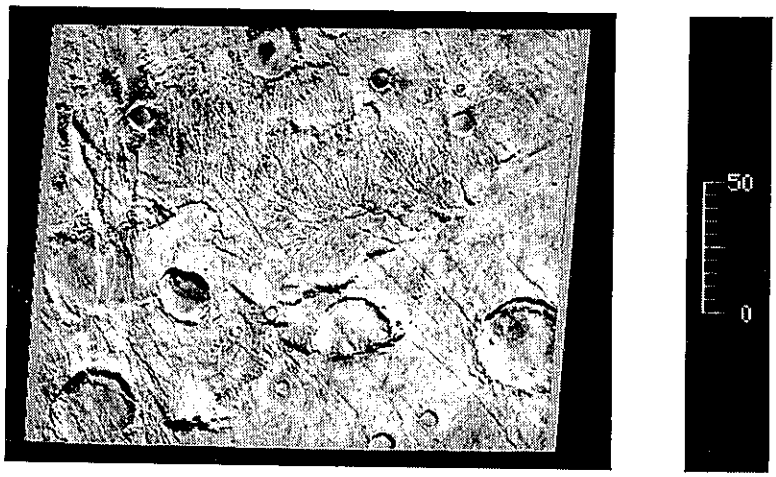

Figure 1. Dendritic valley system in the ancient cratered terrain (48 S, 98 W; Viking frame 63A09, 250 km across). Valleys such as these are found in the ancient terrain and are most probably formed by surface water flow. The source of the water may have been precipitation or subsurface melting. This evidence for the existence of liquid water habitats on the martian surface is the primary motivation for considering the possible origin of life on Mars.

the mean surface temperature fell to the freezing point [10]. This model is based on the persistence of liquid water under ice covers even for average annual temperatures that are below freezing. Such lakes are found on Earth only in the Antarctic [10]. In the dry valleys of the Antarctic deep lakes (30 m) persist under relatively thin (5 m) ice covers. The occurrence of summertime temperatures above freezing provides the energy source, in the form of latent heat of liquid water, that keeps the lake water liquid despite mean temperatures of -20 C. On Mars, modeling studies suggest [9] that the same phenomenon could allow lakes on Mars until temperatures fell to about -35 C --- extending the time for habitability by 500 million years.

Because liquid water could be maintained under thin ice covers long after the rest of the surface would be too cold to support life, lake environments provide an excellent candidate location for searching for fossil evidence of past life. In addition, the sediments that form on the bottom of the lake provide a mechanism for preserving fossil evidence of any life in the lake. Both in respect to the sustenance of life and the preservation of fossils, paleolakes on Mars are likely targets for a search for fossils.

There are several locations on Mars where paleolakes have been identified. One of the best of these sites is Hebes Chasma. This is a box canyon located near the martian equator. Within this canyon is a large plateau the sides of which exhibit layering suggesting deposition in standing water. McKay and Nedell [11] have suggested that the entire plateau is composed of carbonate materials that precipitated when water filled this canyon. These sediments would be ideal targets for a search for organic material and mineralized fossils remains of any early life.

We can consider the following steps on future missions as one implementation of the strategy for searching for life on Mars. First, the evidence that Mars once had stable liquid water on it surface must be confirmed. The date and duration of the major epoch during which water flowed must be established by geomorphological and geochemical methods. Ultimately this question may require a sample return mission. Secondly, given that the presence of water is confirmed, then it is essential to locate sediments that were deposited in standing bodies of water. Such sediments are the most likely locations for preservation of organic and fossil remnants of early life. Once sedimentary locations are identified it is likely that samples for organic analysis will need to be acquired from well below the surface on account of the presence of

oxidants in the top layers of the soil. Sample analysis must also include tests for carbonate and nitrates. Finally, samples could be analyzed for the presence of microfossils --- a task that will almost certainly require returned samples.

EARTH ANALOGS

Perhaps the best way to understand how life might have survived on Mars as the conditions deteriorated is to study life on Earth in Mars-like environments. The most Mars-like place on Earth is found in the dry valleys of Antarctica. Here the mean annual temperature is -20 C and the precipitation is less than in many hot deserts [12]. Despite the cold dry conditions there are two significant microbial communities in these valleys. On the bottom, and in the water column, of the ice-covered lakes, discussed above, algal mats are found. The algae photosynthesize using the low light levels that penetrate the thick ice covers. Further up the mountains the temperatures are cold enough that, even in summer, air temperatures are below freezing. Here the only life is that found within certain sandstone rocks. These cryptoendolith microbial communities survive in the interstitial spaces of the rock but close enough to the surface to receive sunlight for photosynthesis. When the sun shines on the rocks their temperature can be as much as 15 C above the ambient air temperature, rasing the rock temperature well above freezing. Any snow present on the rock surface melts and enters the porous rock, which acts as a moisture trap retaining the liquid water long after the surface has dried.

Using these analogs to life on early Mars we can propose the following scenario for the major steps in the biological evolution on Mars. First, before 3.8 Gyr ago, Mars was experiencing significant accretional input which maintained the thick atmosphere and liquid water. During this epoch Mars was similar to the Earth and it is reasonable to consider that life arose on Mars in a manner similar to the way it arose on Earth [12]. With time the mean annual temperatures fell below freezing. However, as long as the summer peak temperatures exceeding freezing ice-covered liquid water habitats could maintain life, similar to the lakes in the dry valleys of Antarctica. Eventually even the summer peak temperatures would fall below freezing and ice-covered lakes would evaporate. Life could still survive in the rock greenhouses of porous sandstone in a manner analogous with the Antarctic cryptoendoliths. In the final epoch, the pressure on Mars would become so low that liquid

water could not exist even if the surface was heated to high temperatures. Life on the surface of Mars would have ended at this stage.

RESTORING LIFE ON MARS

If further investigation continues to suggest that Mars once enjoyed a more earth-like state with the presence of liquid water, a thick carbon dioxide atmosphere, and even life, then it is interesting to ask under what conditions this habitable state can be restored. Recently, this process, known as terraforming, has been studied seriously using climate models developed for the study of planetary evolution [13]. The main step required for terraforming Mars is warming it to temperatures high enough that hardy microorganisms can survive. One way to accomplish this is to introduce into the martian atmosphere a suite of strong greenhouse gases such as CFCs, ammonia, nitrous oxide, and methane. These gases would warm the surface which, in turn, would result in the release of any carbon dioxide frozen in the polar caps or adsorbed into the regolith. This would accelerate the warming process and within a short time period (100's of years), Mars could have a thick carbon dioxide atmosphere once again. Such an atmosphere would be suitable for many plants and microorganisms but not for humans or animals. The transformation of the carbon dioxide atmosphere into an oxygen-rich one would be lengthy. Plants are the most likely method for producing oxygen on a planetary scale and assuming the typical efficiency of earth's biosphere it would take of order 100,000 years to produce a breathable atmosphere.

Oxygen alone does not make a breathable atmosphere and sufficient nitrogen would have to be included. The amount, distribution, and form of any nitrogen containing sediments is currently the most important question regarding Mars in the context of terraforming.

CONCLUSION

Mars is the world that beckons us in our search for life outside the earth. The life we find there may be long since dead but it would be an exciting confirmation of our theories that life is a natural emergent property of earth-like planets. In addition Mars provides a glimpse of what the future might hold for life and the role that humans --- as the intelligent stewards of that life --- might play in spreading life beyond earth.

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火星の生命探査：
なぜ、どこを、なにを、そして、いかに

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要旨

火星の生命探査を行う前に、その存在の可能性を充分に吟味しなくてはならない。火星の地質学的な変遷および過去の気候の分析から、火星ではかつて生命が発生し、現在においても生き延びていると推測することができる。火星の生命探査のために重要となる探査項目を考察する。また、火星生命探査のシナリオについて比較分析を行うが、数十年もかかるゆっくりした探査より、単一のミッションを緊急に行うことを提案する。

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SEARCH FOR LIFE ON MARS WHY, WHERE, WHAT, AND HOW

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(Received January 24, 1996, Accepted November 5, 1996)

Abstract

Before planning any search for life on Mars, the possibility of its existence should be carefully sought. The survey of the geological history and the past climate of Mars leads to the speculation that life might have happened in the past and survive even until the present day. More than ten important targets of search for such life form are listed. The strategy scenarios are discussed, however, especially, a fast and simple strategy is emphasized.

Key Words: Mars, life, search, water, carbonate

WHY?

Why should we search for life on Mars? The answer depends solely on the question if there were or are any life forms on Mars[1, 2].

Life in the past

Geological record of Mars. The chronological ages on Mars are classified according to its geological ages, which are Noachian, Hesperian, Amazonian in the order from older era to newer era[3-6]. This geological classification is consistent with the actual Martian era, however, based with geologists' interpretations we see many substantial variations. If, in analogy with Earth, Life has begun 3.5 to 4.0 billion years ago on Mars, its geological classification falls into Noachian or Hesperian. The area where Noachian or Hesperian are preserved on the surface lies mostly in the highlands of the southern hemisphere of Mars. *Lunae Planum* and *Syria Planum* are representing this geological era, and so the *Tharsis Mons*. The area in the northern hemisphere where fewer numbers of craters are found is a relatively newer layer, and does not seem to preserve the record of the era 3.5 to 4.0 years ago.

Were organic compounds formed on Mars? Yes or No: It is reasonable to assume that the Martian life form, if any, is composed of organic compounds. Assuming any life form had originated on Mars, there must have been an abiological formation of abundant organic compounds at least local area in the same manner as the terrestrial case. In this case, the existence of liquid water is required. Assuming Mars was once warm, the abundant amount of organic compounds might have been formed because of the existence of ocean or river with an energy source such as lightning. The organic compounds, however, are not presently found on the surface or in the atmosphere. There is probability that they will be found underground or in the deeper layer of Polar Caps. The finding of organic molecules will be the bottom line for the search for life.

Was there water on Mars? Yes: Water is essential for the birth of life, and the existence of water is critical for the existence of life on Mars. The soil analysis by Viking, the measurement of the atmosphere, the observation of Polar Cap, and the analyses of SNC meteorites all prove the existence of water in the present era. The quantity, however, sounds deficient for maintaining biological activity. The next question is: Was there abundant water in the past?

Was there ABUNDANT water on Mars? The present-day analyses of morphology of the Martian surface suggest there was undoubtedly water on the surface. *Outflow Channels,* which appear as rivers, are not considered to be formed by water flow. On the other hand, the formation of *Valleys* is attributed to water, either as spring water from

underground or from precipitation such as rainfall[7]. The *Valleys* are only found on the ancient layers and their formation is assumed to be completed by 3 to 4 billion years ago. The *Valleys* are also only found at the lower latitude regions up to 65 degrees and are rarely found around the Polar regions. Their typical width ranges from less than 1 to 10 kilometers and the length for less than 5 kilometers to 1000 kilometers. The *Valley* is a sole direct evidence for existence of water at the beginning of the Martian history, and will be the first candidate for the site for future search for life.

Was Mars once a warm planet? Yes or No: There are still numerous discussions about this issue[8]. Assuming Mars once had a warm climate, life would possibly have originated as analogy with the case of earth, If Mars was not warm, on the other hand, some special mechanisms should be sought. There is the report suggesting the surface temperature exceeds the freezing point if Mars had CO_2 rich atmosphere of 1-5 atm. In this case, many carbonates would remain on the surface, The search for carbonate, thus becomes extremely important to determine the ancient climate of Mars.

Life in the present

Is there life in the present? Yes or No: The condition of Martian surface is extremely harsh and life does not appear as we can recognize in the present day, the survival of microorganisms underground, however, may be probable.

Where?

The area we recommend for search will be:

The area where the geological layers are 3 to 4 billion years old. As noted earlier, life might have originated during this era and its probability is the highest throughout the Martian history. The geological term for this era is Noachian or earlier Hesperian. Such geological layers are mainly preserved in the southern hemisphere, and are also found in *Lunae Planum*, *Syria Planum*, or *Tharsis Montes*.

The area where water was abundant. These are the regions around valley networks, which are widely located between the latitudes 0 and 65 degrees.

The area where volcano was active. *Tharsis Montes*, one of the examples, are also believed to be 3 to 4 billion years old.

The area where bio-organic compounds are accumulated and preserved. The *Polar Cap* region, either northern or southern, is an accumulated ice-layered structure, and composed of frozen H_2O and CO_2 as well as aelion dust (fine soils, sediments) which are accumulated residues resulting from sand storms. The top surface layers are as young as 10^7 years old, however, the layers have been accumulated for 3 billion years[9]. The systematic analyses of the longitudinal sections of such layers will provide information on materials that existed on Mars in the past. In the valley of *Polar Cap* the ancient layers are exposed so that the old sample can be directly examined. The present low temperature at this area seems to prohibit the occurrence of life, although, it is probable that organic molecules, which might have been abundantly formed in the past warm era, could have migrated and have been preserved in these ice layers.

What and How?

Water. Water is essential for formation of bio-organic molecules, evolution process of life, and biological activity in any life body. Viking landers found water in the atmosphere, on the surface (as frost), and in fine soil (1 to 3 %). The biggest question here is whether there is evidence indicating the existence of abundant water in the past era. For the future search for water, γ-spectroscopy, direct search with a penetrator or a rover, or seismic search will be used.

Water in the past. The research on surface morphology revealed that there might have been water, at least flooding water, on the past Martian surface, as discussed in the previous section. We need to know, however, whether water was abundant or not.

Electrolytes. Even the simplest terrestrial life forms require electrolytes. The existence of K, Mg, Ca, Cl, and S in Martian soil is already documented, and Na and P are also expected.

Nitrogen. Nitrogen in any form is not found on Mars.

Carbonate. If there are abundant carbonate minerals, it might prove Mars had a warm climate or CO_2-rich atmosphere in the past.

Carbonates have some absorption band in IR, which has not been detected by remote sensing[10]. The SNC meteorites contain a small amount of carbonate (0.3 % of the bulk meteorites) by their evolved gas analysis[11]. Further detailed examination is truly required.

Carbon-bearing molecules other than CO or CO_2. CO and CO_2 are abundant on Mars, but the other carbon-bearing molecules that are more reducing than CO or CO_2 need to be detected to verify biological activity.

Trace gases. The abundance of oxygen found in the Martian atmosphere is 0.13%, and methane is estimated to be as little as 0.02 ppm at most if it exists. The amounts of these trace gases should be accurately documented; it is also interesting if local evolution of these gases is found.

Isotopic ratios. The isotopic ratio of carbon in the terrestrial carbonate minerals differs from a natural ratio due to biological activity. The value $\delta C(‰)$ is roughly -25 in the terrestrial case, whereas the preliminary analysis of SNC meteorites indicates this value ranges from somehow 0 to 10. The detailed *in situ* examination using an actual Martian soil will determine this value.

Clay minerals. The reasons for the importance of clay minerals are two-fold. The existence of clay minerals will determine whether there was abundant water in the Martian history. Most clay minerals are formed through chemical weathering processes or aqueous alteration. Another feature of clay minerals is the possible catalytic role for a stage of chemical evolution. As some school of Origin of Life research argues, if life takes advantage of clay in the process of its own chemical evolution, the same relation between life and clay is most likely to persist on Mars.

Volcano in the past. If Mars once had a warm climate, different kinds of energy sources would be available for prebiotic processes. On the other hand, assuming Mars was never warm, the only available energy source would be volcanic activity. A volcano usually emits H_2, H_2S, SO_2, CH_4, CO, and CO_2, and a search for these trace gases will help solving this issue.

Microfossil. Microfossil is a direct evidence for life, but will be difficult in unmanned mission.

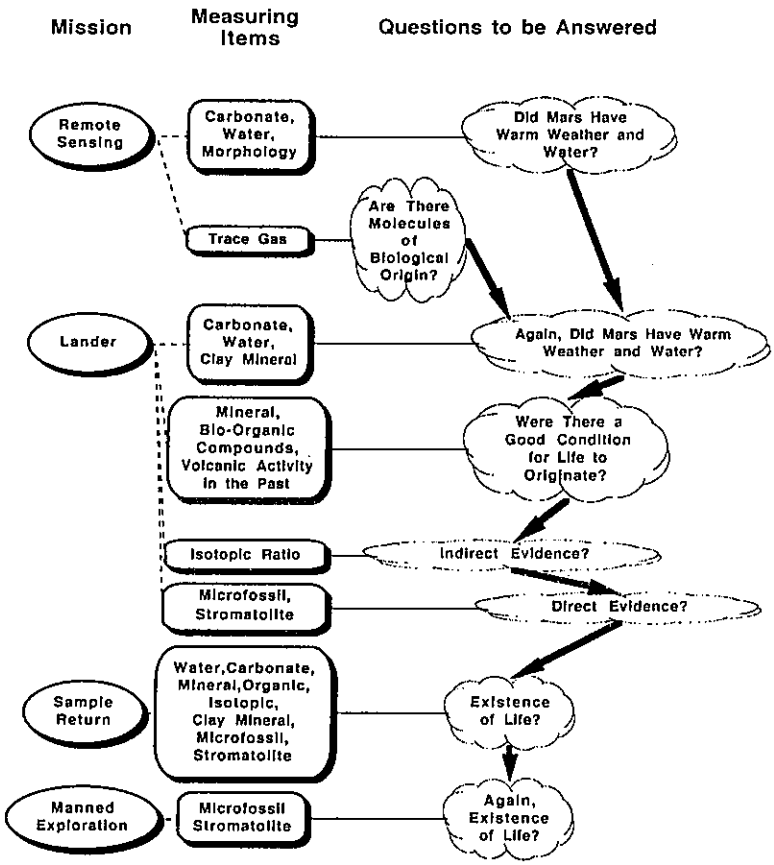

Figure 1. Logical Step-by-Step Strategy for Mars Exobiology Mission

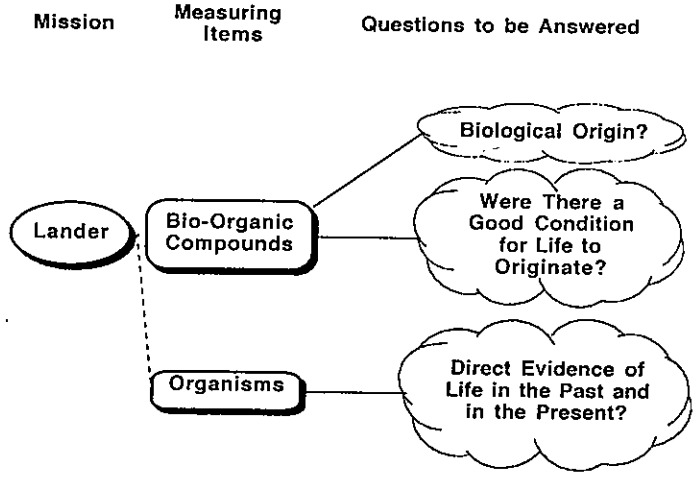

Figure 2. Quick Step for Mars Exobiology Mission

Stromatolite. If photosynthetic microorganisms ever originated on Mars, there will be a good possibility to find stromatolite. Non-phototactic microorganisms, however, do not form stromatolite.

Bio-organic compounds. Viking landers failed to detect any organic compounds on Martian surface. Underground soil or polar cap regions are candidate sites for search for any bio-organic compounds.

Microorganisms. The process of chemical evolution does not seem to be occurring in the present era, however, there is still possibility for ancient microorganisms to survive in some niche.

Strategy

Based on the search list discussed in the previous section, the logical strategy of search for life on Mars is speculated as Figure 1. In this case, the missions are supposed to continue step-by-step from remote sensing to lander, and then to sample return mission[12]. As shown in this diagram, the "logical strategy" is a steady, however, slow step; it seems to be harder to go on in the current budget situation of major space faring nations. Instead, we might take a completely different approach toward such mission like Martian exobiology mission. We cannot afford many successive missions, so we should pursue a direct search for life on Mars. This type of mission strategy is shown in Figure 2. The organic molecules, the essential ingredients of life, and microorganisms, the simplest form of life, are two targets of search. Although site selection and instrument development require tremendous work in advance, we need such fast and simple mission. If it concludes as a success, its impact will furnish invaluable answers.

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火星上での生体有機化合物の生成とその検出

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要旨

火星に生命や有機物が存在するかどうかはまだわかっていない。例えばヴァイキング計画においては火星表土中に生命や有機物は検出されなかったが、一方、火星隕石中に生物由来の有機物の存在の可能性も示唆されている。ここでは、火星上にどのような有機物が存在する可能性があるかをまず議論する。初期火星大気は初期地球と類似した二酸化炭素・一酸化炭素・窒素・水などからなる組成を有したと考えられる。このような混合気体に宇宙線成分である高エネルギー粒子線を照射するとアミノ酸前駆体などの生体有機物が生成する。また、彗星や隕石などにも種々の有機物が検出されており、これらも火星有機物の供給源と考えられる。地球上の生命がこのような地球由来の有機物と地球圏外有機物をもとに誕生したとするならば、同様なことが火星で起こった可能性が考えられる。その場合には火星生物由来の有機物の存在が考えられる。このような火星有機物の検出法についても議論する。

Viva Origino 24(1996)277－292

FORMATION AND DETECTION OF BIOORGANIC COMPOUNDS ON MARS

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(Received December 1, 1996, Acceted December 18, 1996)

ABSTRACT

It is probable that early Mars had the same type of atmosphere as early Earth. If so, major components of primitive Mars atmosphere are CO_2, CO, N_2 and H_2O. Irradiation of high energy particles to such a gas mixture caused the formation of amino acid precursors. It is believed that life was born on the earth using such endogenously-formed organic compounds, with exogenous organic compounds carried by comets. We can expect that organic compounds could have been formed on the primitive Mars, though the Viking results did not show the presence of organic compounds on Mars. If we choose the other sampling sites and/or the other analytical methods than those of the Viking Project, organic compounds may be detected. Possible organic compounds on Mars and analytical methods for them are discussed.

Key words: Mars, life, organic compounds, cosmic ray, amino acids, Viking mission, Return to Mars Together, mass spectrometry

Introduction

Has life ever started on Mars? Is there life on Mars? It is still controversy question. Although the Viking missions, which landed on Mars in the 1970', found neither bioorgaic compounds nor organisms on Mars, it is difficult to exclude the existence of life on Mars [1]. Because, the soil samples, which were collected from the Martian surface, might be irradiated by ultraviolet sunlight and might be strongly oxidizing soils.

Now it is suggested that early Mars had the same type of atmosphere as early Earth, which is a mixture of carbon dioxide, carbon monoxide, nitrogen and water [2]. Irradiation of high energy particles to such a gas mixture caused the formation of amino acid precursors[3-5]. On the Earth, life was generated using such endogenously-formed organic compounds, with exogenous organic compounds carried by comets[6] about 4 billion years ago. We can expect that organic compounds could have been formed on the primitive Mars.

In Viking mission, several biological investigations were conducted, including detection of organic compounds in Mars regolith [5]. A pyrolysis-gas chromatograph/mass spectrometer (PY-GC/MS) was applied to organic analysis on Mars; no more than trace amounts of organic compounds were found by the technique [7]. Those results do not necessarily show that there are no organics at any place on Mars. It is now believed that water was abundant on the primitive Mars, and that a part of the water is still preserved as underground frozen soil. If we choose the other sampling sites and/or analytical methods than those of the Viking Project, organic compounds may be detected. A new project called *Return to Mars Together* is now under consideration [8]. Here possibility of the formation of organic compounds on primitive and recent Mars is discussed, together with analytical methods for organic compounds on Mars.

POSSIBLE ORGANIC COMPOUNDS ON MARS

There are several possible origins of organic compounds. The Viking results showed that surface regolith of Mars did not contained any classes of organics since it contained superoxides and peroxides which might destroy any organic compounds there. Organic compounds on Mars may, however, be present in the place without the oxidants: Ices in polar caps and underground frozen soils are two possible candidates. Table 1 summarizes some possible origins of organic compounds on Mars, together with possible sites where they might be found.

Table 1. Possible sources and sites of organic compounds on Mars

Sources	Sites	
	Uuderground	Polar caps
(i) Organic compounds *abiotically* formed from primitive Mars atmosphere	○	
(ii) Organic compounds delivered out of Mars, *e.g.*, Cometary organics	○	△
(iii) Organic compounds *biotically* formed by Mars organisms	○	
(iv) Organic compounds *abiotically* formed from the present Mars atmosphere.		○

Organic compounds *abiotically* formed from primitive Mars atmosphere

Numerous studies have been conducted to show that bioorganic compounds such as amino acids are easily formed in a simulated primitive earth atmosphere. In these experiments, such energies as spark discharges [9], ultraviolet lights [10] heat [11], shock waves[12] were used to promote organic formation. It is, however, strongly suggested that the primitive atmosphere of the earth was composed of carbon dioxide, carbon monoxide, nitrogen and water [2], that is, the mildly reduced gases. It is not easy to form amino acids from these slightly reduced gases by irradiating discharges [13,14] or ultraviolet lights[15].

Kobayashi et al.[3-5] showed that amino acids or their precursors are easily produced even in mildly reduced gases by irradiating with high energy charged particles. A wide variety of amino acids was found in all the hydrolysates of irradiation products, when carbon monoxide and nitrogen were used in the starting materials. Glycine was predominant, followed by aspartic acid, serine, alanine and ß-alanine. The D/L ratios were measured as ca. 1 by GC.

The amount of products was in proportion to the total energy deposited, and is independent of the kinds of irradiated particles (protons, helium nuclei or electrons) as well as of initial energy. This indicates that not only primary cosmic rays but also secondary particles, nuclear and electro-magnetic cascades generated in the atmosphere, would contribute the abiotic synthesis of amino acid precursors. Cosmic rays would contribute the formation of organic compounds until they lose

their kinetic energies. The G-values (number of formed molecules per 100 eV) of glycine were obtained as about 0.02 when a 1:1 mixture of carbon monoxide and nitrogen was irradiated with protons. It was shown that high energy charged particles as cosmic rays are a more effective energy source for prebiotic synthesis of amino acids than conventionally considered energy sources like spark discharges and ultraviolet light. The energy flux of cosmic rays at 0.011 cal/cm^2 yr 2π which is given by integrating from 10^9 eV to 10^{12} eV including heavy cosmic ray nuclei (calculated after Meyer et al.[16]). This energy deposit corresponds to the production of glycine of 1.0 μmol/m^2 yr in the case of a 1:1 mixture of carbon monoxide and nitrogen.

As mentioned before, early Mars had the same types of atmosphere as that of early Earth, together with liquid water. Energies such as cosmic rays should have provided not only to early Earth, but also to early Mars. Thus it is plausible that, if organic compounds were formed on early Earth before the birth of life, the same kinds of organic compounds were formed on early Mars. If living organisms (particularly, heterotrophs)were generated on early Mars, these abiotically-formed organic compounds should have been rapidly consumed by them. On the contrary, if life was not born on Mars, these abiotically-formed organic compounds might have been preserved in some Mars environments such as in underground frozen soils.

Organic compounds delivered out of Mars

Organic compounds have been detected in such extraterrestrial environments, such as in interstellar space, in Jupiter, in Titan, and in comets. When various kinds of gas mixtures were irradiated with protons of 2.8—4.0 MeV, amino acids were detected in hydrolysates of every type of gas mixtures, if proper carbon compounds (carbon monoxide or methane) and nitrogen compounds (nitrogen or ammonia) were in the starting gas mixtures. Even if there is no water in the starting materials, amino acid precursors, which give free amino acids after acid hydrolysis, were produced. This fact indicates that bioorganic compounds could be formed by comic radiation wherever proper carbon compounds and nitrogen compoundswere available.

Among organic compounds found in space, those in comets have been most interestng from the point of view of origins of life on Earth-type planets since they could be directly delivered by collision of comets to the planets and/or in the form of micrometeorites[17]. Comets are believed to be aggregates of interstellar dust particles (ISDs). There have been several works on formation of organic compounds in ISDs. When a ice mixture of methane (or propane), ammonia and water was irradiated with high energy protons, several kinds of amino acids were detected in the hydrolysates of the resulting non-volatile residues recovered on the metal block [6, 18]. It was shown that amino acid precursors can be formed by

irradiation of not only gas mixtures but also ice mixtures with high energy particles, if proper carbon and nitrogen sources are contained in them. It is suggested that there are amino acid precursors in cometary environments, though free amino acids have not been detected there[19].

Carbonaceous chondrites have a wide variety of organic compounds. Most of organic compounds are believed to be complex polymers, and such organic compouns as amino acids [20] have been found in pyrolytic products or solvent extracts from them.

These extraterrestrial organic compounds could have been supplied by comets, asteroids, meteorites, and interplanetary dusts. It is plausible that, as well as endogenous organic compounds, exogenous organic compounds dissolved in primordial Mars ocean about 4 Ga, and a part of them have been preserved in underground frozen soils.

Organic compounds *biotically* formed by Mars organisms

Though the results of Vikig missions in 1976 could not show the presence of life on Mars, it is still possible that there are/were organisms in certain environments in Mars. McKay and coworkers[21] reported that meteorite ALH84001, which was formed about 3.6 Gyr ago in Mars and deliverd to Antarctica of Earth about 13 kyr ago, contained possible relic biogenic activity.

In the case that life was generated on primitive Mars, biogenic organic compounds should have been released into Mars environment both when they alive and after they died. Besides them, organsms themselves should be good targets to be detected. If they have died, we cannnot use the life-detection techniques used in Viking Missions. Recently Tsuji et al. [22] proposed new detection system for organisms called "microscopic fluorometry method." By the method, not only living organisms but also "dead organisms" can be detected.

Organic compounds *abiotically* formed from the present Mars atmosphere

The composition of the atmosphere of present Mars surface is reported as followes (Owen et al., 1977) : CO_2 (95.32 %), N_2 (2.7 %), Ar (1.6 %), O_2 (0.13 %), CO (0.07 %), H_2O (0.03 %). It is possible that organic compounds including amino acid precursors are formed from a gas mixture containing carbon monoxide, nitrogen and water by action of cosmic ray or ultraviolet light[23]. Since molar ratio of carbon monoxide nitrogen and water are small, expected formation rate of organic compounds is small. Most of the organic compound produced from the present Mars atmosphere would be adsorbed onto Mars surface, and would be decomposed by superoxides and peroxides in Mars regolith, as implied from the Viking results. The organic compounds formed in polar areas could be, however,

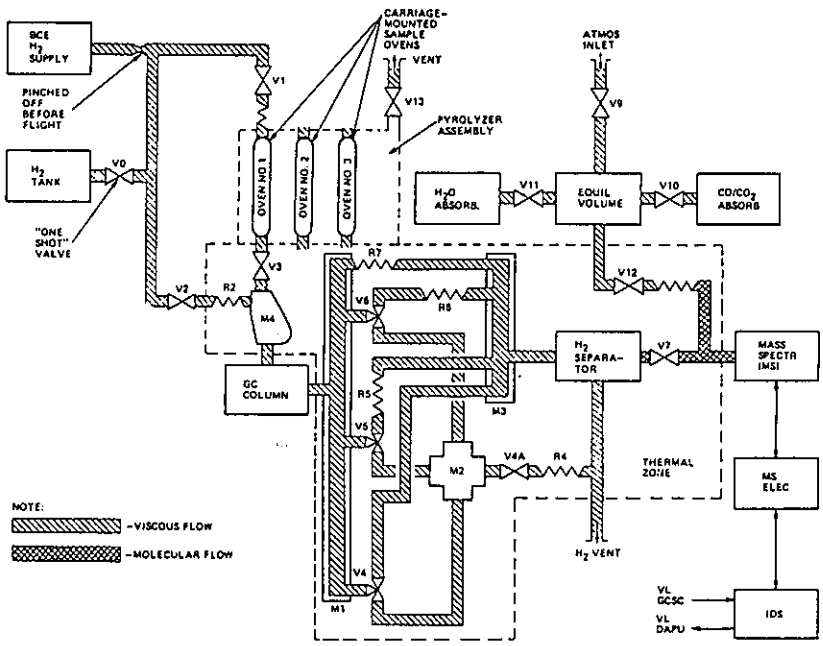

Fig. 1. Gas Flow Block Diagram of the Viking Pyrolysis-GC/MS instrument.
Dotted line delineates the heated zone, and M1, M2, V4-6, R4-7 are the effluent dividers. Cited from Ref. 25.

trapped in water ice or dryice of polar caps. A part of ice is sublimed in summer, and organic compounds in it could be condensed year by year. Thus organic compounds in polar ices will be an interesting target to be analyzed.

DETECTION METHODS FOR ORGANIC COMPOUNDS ON MARS

Here we discuss several detection methods for the organic compounds on Mars used in the past studies and those which might be used in the future Mars mission [24].

Pyrolysis (PY)-GC/MS

The PY-GC/MS technique was applied to the analysis of organic compounds in Mars surface regolith in NASA's Viking Mission in 1976 (Fig. 1). A major advantage of PY-GC/MS is that both solid and liquid samples can be introduced to the system without any pretreatments. In ground experiments, carbonaceous chondrite was analyzed by the same method, which gave many peaks of organic

compounds such as benzene[25]. Significant amounts of organic compounds were, however, not detected. The reason no organic compounds were detected are: (i) Mars regolith analyzed in Viking Mission was highly oxidized, and/or (ii) PY-GC/MS is not a suitable analytical technique if the nature of Mars organic compounds is different from that of carbonaceous chondrites. If only sampling sites are the ploblem, PY-GC/MS could be applied to the samples in ices or frozen soils.

Resently the PY-GC/MS is appllied to such samples as whole microorganisms. For example, Holzer *et al.* [26] analyzed microbial fatty acid constituents by PY-GC/MS, where fatty acids were methylated during pyrolysis with trimethylanilinium hydroside. Addition of such reagents for derivatization may be useful for analysis of Mars organic compounds.

A mass analyzer of sector-type was used in the Viking GC/MS. If there is a strict limitation for weight of the system, other types of mass analyzers, including an ion trap and a quadrupole mass analyzer are of choice with the PY-GC/MS.

Characteristic of organic compounds on Mars are strill unknown by now. It is important to test the PY-GC/MS with various kinds of samples including the products of simulation experiments with simulated Mars soils[27], with and without adding some reagents before pyrolysis.

Mass spectrometry with soft ionization methods

If organic compounds are to be analyzed without decomposition, mass spectrometry techniques with so-called "soft ionization methods" are of choice, which include Liquid-Secondary Ion Mass Spectrometry (LSIMS), Fast atom bombardment Ionization(FAB)-MS, Laser Desorption Ionization (LD)-MS and Matrix-Assisted Laser Desorption Ionization (MALDI)-MS. By these methods compounds whose molecular weights are thousands and more can be analyzed [28]. Organic compounds in geological samples should usually be extracted with water or other solvents before analysis.

Choice of ionization methods and types of analyzer depends on the amounts and ranges of molecular weight of the target molecules. If the molecular weight range is wide (i. e., up to thousands dalton), amounts of individual molecules are low. It is difficult, in such cases, to detect molecular ion peaks of the samples. If major molecular weights of the sample are several hundreds, it will be easier to measure molecular weights of the samples. Here we consider the latter case.

Engelke et al. [29] developed a new LD-MS system using both infrared and ultraviolet laser pulses named Two-Step Laser Mass Spectrometry (L^2MS). Fig. 2 shows the procedures for the system. A part of sample solution on a solid substrate was desorbed by an CO_2 laser pulse (10.6 μm) ; most of the desorbed molecules are neutral (not ionizized). Then the second laser pulse, usually a Nd: YAG laser (266 nm) is used, was irradiated to the desorbed molecules to ionize

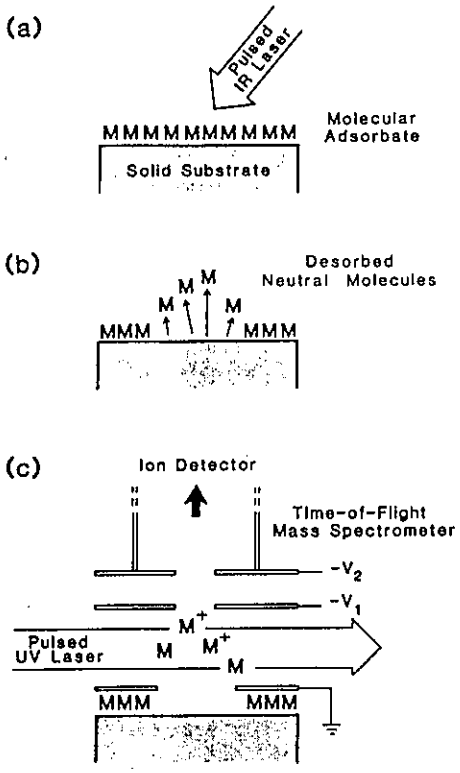

Fig. 2. Ionization procedure for L^2MS system. Cited from Ref. 32.
(a) Molecular adsorbated on a solid substrate are irradiated with CO_2 laser pulses.
(b) Neutral molecules are desorbed from the substrate.
(c) Desorbed neutral molecules were ionized with a Nd:YAG laser (266 nm) after a suitable time delay, and ions are analyzed with a time-of-flight mass spectrometer.

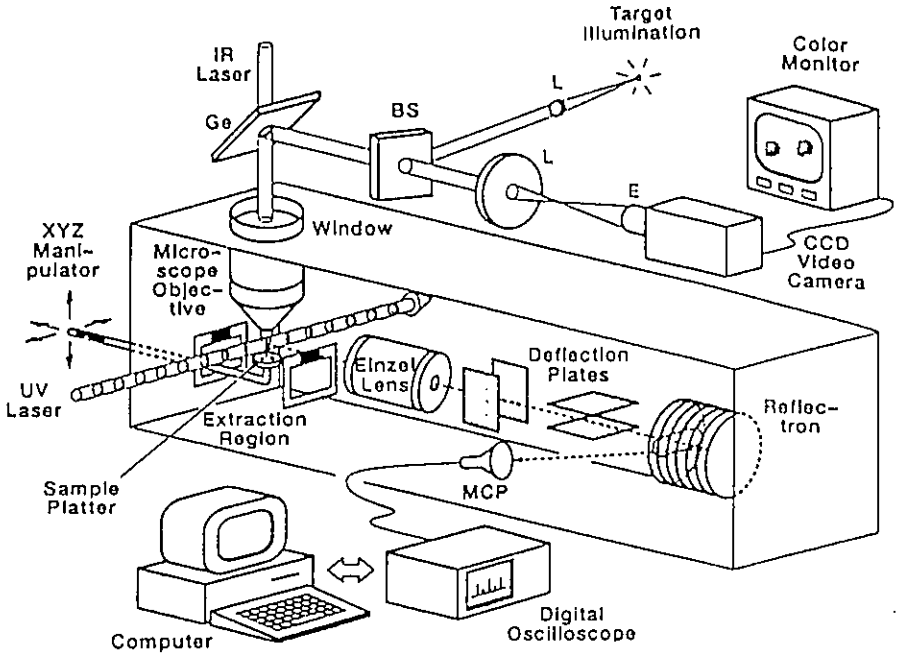

Fig. 3. Schematic Diagram of a μL^2MS system. Cited from Ref. 30.

them after a suitable time delay. The formed ions are analyzed with a time-of-flight mass spectrometer (TOF-MS). When the Nd-YAG laser was used, compounds with aromatic rings, such as polyaromatic hydrocarbons (PAHs) and nucleic acid components, could be analyzed in high sensitivity and in high selectivity.

Kovalenko et al. [30] modified the L^2MS system for microscopic organic analysis: The system was named μL^2MS (Fig. 3). They analyzed PAHs in carbonaceous chondrites and micrometeorites. McKay et al. (1996) used the present system for the analysis of Martian meteorite ALH 84001, and reported there are PAHs in it. This observation, together with analysis of carbonate and magnetite in the same sample, led them to conclude that they are evidence for primitive life on early Mars, though their conclusion is still controversial.

The L^2MS method is insensitive to the compounds without aromatic rings, such as amino acids and peptides unless wavelength of the second laser pulse is quite short. For the analysis of such compounds, other types of mass spectometers could be chosen. Successful analysis of small peptidcs of several hundreds dalton were reported by using a matrix-assisted laser desorption ionzation (MALDI) ion source coupled with an ion trap and a time-of-flight (TOF) mass analyzer (Fountain et al., 1994). MALDI-TOF-MS analysis of some simulated Mars samples is one of the candidate to analyze Martian organic compounds.

Amino acid analysis

If we concentrate on detection of "bioorganic compounds" in Mars samples, amino acids should be analyzed, since amino acids are more easily formed abiotically than nucleic acid bases and sugars. The sample must be hydrolyzed because not free amino acids but amino acid precursors are likely to exist in abiotic environments.

Subpicomole level of amino acids can be detected by fluorometry after derivatized with some reagents such as o-phthalaldehyde and thiol compounds. Amino acids may be analyzed by GC or GC/MS, which requires derivatization (such as trimethylsilylation) before injection. The main disadvantage of these amino acid detection methods is the analytical procedures requires several steps; extraction, hydrolysis, reaction(s) with reagents and, occasionally, desalting.

Detection of cyanides and aldehydes

No organic compounds have been identified on Mars *in situ* by far. The simplest and important organic compounds for life is hydrogen cyanide (HCN) and formaldehyde (HCHO) since they are intermediates for abiotic formation of amino acids, purines and sugars. HCN can be detected with an ion-selective electrode, and HCHO can be detected fluorometrically after derivatization.

It will be difficult to detect HCN and/or HCHO which is formed in primitive

Table 2. Detection methods for organic compounds on Mars

Target	Method	Pre-Treatment	Source of Organics*			
			a	**b**	**c**	**d**
1. Organisms	Fluorescence microscope	Reagent addition			○	
2. Complex organics	Pyrolysis -GC/MS	None		○	△	
		Reagent addition	○	○	○	
	MS with soft ionization (*eg.* MALDI-MS)	Solvent extraction	△	△	○	
3. Amino acids	Fluorometry	Hydrolysis /Derivatization	○	○	○	△
4. Simple compounds:						
HCN	Ion electrode	Water extraction				○
HCHO	Fluorometry	Water extraction /Derivatization				○

* **a** Organic compounds *abiotically* formed from primitive Mars atmosphere;
b organic compounds delivered out of Mars, *e.g.*, Cometary organics;
c organic compounds *biotically* formed by Mars organisms;
d organic compounds *abiotically* formed from the present Mars atmosphere.
○ and △ are evaluation at the present stage.

Mars or extraterrestrial environments since they are very reactive and are easily polymerized. Thus the first choice in sampling sites on Mars to detect them are polar caps. The present Mars atmosphere has a trace amount of carbon monoxide, nitrogen and water in it, and they will react to form HCN and/or HCHO by the acition of cosmic rays (Kobayashi *et al.*, 1993). Freshly-formed HCN and HCHO may be preserved in water ice or dryice of the polar caps.

CONCLUSION

McKay and coworkers' report that Mars meteorite had a trace of primitive life in early Mars is controversial, and further researches is strongly required. It may now give an another chance of Mars exobiology missions. Thus it is quite necessary to develop new analytical instruments on board Mars landers.

Table 2 summarizes detection methods for organic compounds of Mars proposed here. In order to determine the detection method for organic compounds on Mars, sources of organic compounds (**a** to **d** in Table 2) should be considered at first. In the case of **a**, **b** and **c**, underground frozen soils are the samples of choice, while are the polar cap ices in the case of **d**. In both samples, organic compounds are expected to be preserved without decomposed by ultraviolet light, cosmic ray, nor superoxides.

Secondly, we need appropriate test samples to build analytical methods for them. Terrestrial organisms should be used in place of biologically-formed Mars organic compounds (**c**). As organic compounds which was abiotically-formed on Mars (**a** and **d**), we have nothing but products of simulation experiments. We chose products formed by proton irradiation of a gas mixture of "simulated primitive Mars atmosphere" (CO_2 + CO + N_2 + H_2O) as test samples. Carbonaceous chondritre was used as the test sample for the Viking PY-GC/MS instrument, but other types of samples such as products of simulation experiments should also be tested as extraterrestrially-formed organic compounds on Mars (**b**).

International collaboration for evaluation of detection methods is now required to establish analytical techniques for "Return-To-Mars-Together." Some common test samples, which contain both simulated Mars organic compounds and inorganic matrix, should be used there.

Acknowledgments: This work was partly supported by Grant-In-Aid (Nos. 06832009 and 07640653) from the Ministry of Education, Science and Culture, Japan.

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地球外生命を探す
—微生物検出法の開発—

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要旨

地球上、地球外における微生物を検出するための方法を開発しているので紹介する。

この方法は蛍光顕微鏡を用い、直接微生物を観察記録するものである。この方法は顕微蛍光法と呼ばれ、自然界の微生物を検出する手段として２０年ほど前から世界的に開発が進められていたが、種々の困難のため一般的手法とはなっていない。我々は新しい色素や検出法を導入することにより、従来の方法よりより効率の良いシステムを開発した。特に蛍光色素としてはエステラーゼの基質を用いることにより、生物と非生物との判定が明確になり,かつ細胞の生死の判定も可能となった。proteinoidのような原始細胞モデルも検出可能になった。

現在、火星の生命探査のための自動小型検出系の構築を進めている。

Viva Origino 24(1996)293－304

SEARCHING FOR EXTRATERRESTRIAL LIFE
- Development of detection system of microorganisms -

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(Received August 17, 1996, Accepted November 7, 1996)

Abstract

A noble method for the exploration of terrestrial and extraterrestrial soil microorganisms, especially targeted for the polar regions and Mars, has been developed. This method is based on the microscopic observation using fluorescence techniques. Microorganisms could be fluorescent by adsorption, enzymatic cleavage of extrinsic fluorophores , and also by intrinsic fluorophores. By combined utilization of these fluororescent probes, we could detect almost all the terrestrial microorganisms examined. Since this method is very sensitive and does not require culture, we believe that it is applicable for exploration of polar and extraterrestrial microorganisms.

Introduction

Considering the history of life on Earth, the most possible extraterrestrial life is microorganism. However, detection of microorganisms is not easy even on Earth. The reasons are that microorganisms are very small (most of them are less than 10 μm) and are often very difficult to distinguish from soil particles and detritus. Besides, culture conditions are not established. When it comes to

extraordinary microorganisms, situation becomes even worse. Considering this situation, we think that the direct detection of microorganisms by using fluorescence microscopic techniques is most promising. Detection of microorganisms in natural environments with fluorescence method has been developed these twenty years (1-12) . In most cases, adhesive fluorescent probes ,such as acridine orange, ethidium bromide, calcofluor white M2R and fluorescent antibodies are used. The fluorescence images were recorded in conventional photographic films. However, these classical methods have many difficulties if we want to get reliable, reproducible and quantitative data using soil specimen, due to non-specific binding of the probes, weak fluorescence and rapid photobleaching. In order to overcome these difficulties, we have developed a new fluorescence method applicable to soil specimen using fluorescent enzyme substrates and a highly sensitive solid state camera (13-15).

We found that our method can effectively distinguish microorganisms from soil particles. If extraterrestrial life is composed of the same kinds of organic molecules as terrestrial life, this fluorescence microscopic technique can detect most of them.

In this review, I would like to describe our fluorescence microscopic technique.

Establish an experimental standard for "How unknown objects are life-like."

Life can be characterized by three major factors, 1) possesses genetic information (nucleic acids), 2) discriminates self and outer world (cytoplasmic membranes), 3) exhibits metabolic activities (enzymes) . Each characteristic organella or molecule is specifically detected by fluorescent probes (Fig.1). We

(photobleaching during exposure, non-linearity in intensity of acquired images), and relative background fluorescence intensity were remarkably reduced compared to classical photographic film imaging. Image processing techniques enabled the rapid and quantitative analysis of the obtained data.

Results

A. The present method detected almost all culturable cells tested (about 50 species). Even spores of bacteria, yeasts, slime molds and fungi were clearly detected. An exception was *Holobacterium halobium* (13-15).

B. When purely isolated microorganisms were mixed with soil, the microorganisms were clearly identified (Fig.2). In Fig.2, bacteria, fungi and molds were mixed with soil and the mixture was stained with three types of fluorescent probes. The objects indicated by a star are stained with all three fluorescent probes, while the objects indicated by arrows are stained only with ethidium bromide. Therefore, the formers can be classified as microorganisms and the latters as detracts or soil particles. Actuary, the star-marked objects are spores of the cellular slime mold.

C. Fluorescent esterase substrates such as SFDA { 5-(and -6)-sulfofluorescein diacetate} discriminated reproductive cells from non-reproductive (13). A membrane probe, ANS (1-anilinonaphtalene 8-sulfonate) and a nucleic acid probe, acridine orange gave poor discrimination (FIG.3).

D. Spores of the fungi possessing dark brown pigments are very difficult to detect even with the probes shown above. Pretreatment of the spores with chemical bleachers such as peroxides or perchlorides greatly improved the detection efficiency (14).

can stain unknown objects with three types of the fluorescent probes. If the objects are stained with the three probes, the score is (***) and they may possibly be microorganisms, and if they are stained with only one probe, the score is (*) and they may possibly be detritus or non-biological substances. Thus, we can make an experimental standard for the definition of Life with fluorescence microscopic techniques. This scoring can be referred to as "Michelin restaurant standard".

To establish " **Michelin restaurant standard**" for life
How the unknown or artificial "*life-like*" objects are "*life-like*".

characteristics of life	molecules and molecular assembly	fluorescence dyes
distiction between self and outer world	cytoplasmic membranes	hydrophobic probes
gradients between self and outer world	pumps and channels on cytoplasmic membranes	potential sentisive probes
metabolic activities	enzymes,electron transport systems	enzyme substrates
self-reproduction genetic information	nucleic acids	nucleic acid probes

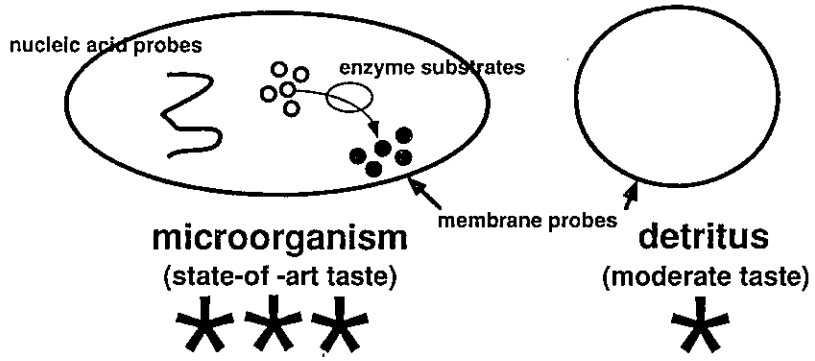

Fig.1. "Standard" for judging how unknown objects are life-like by fluorescence microscopic technique.

Apparatus

The present method employed a highly sensitive cooled CCD camera and highly microorganism-specific fluorescence probes. Due to these improvements, very weak fluorescence could be detected, and undesirable photo-effects

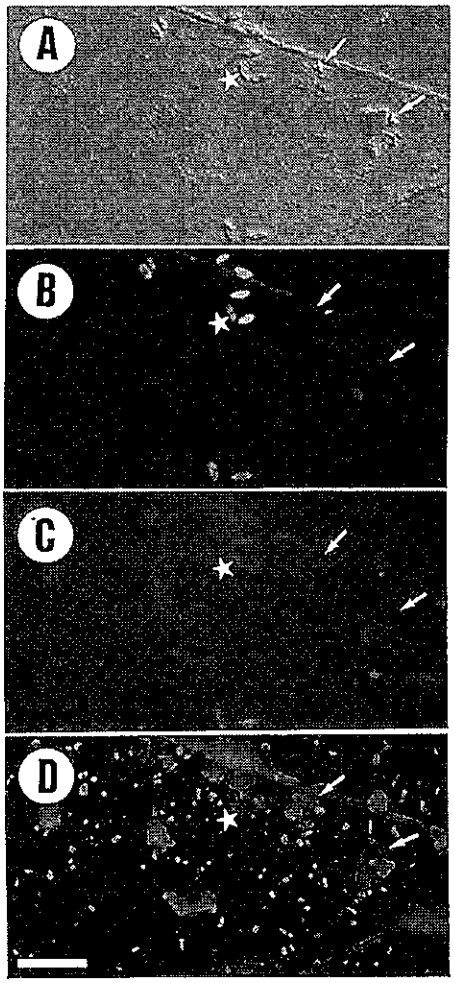

Fig.2. Triple staining microscopic images of the mixture of microorganisms and soil.

Cultured *Escherichia coli*, slime mold and fungi were mixed with soil and stained with ANS , CFDA-AM and EB.
ANS:1-anilinonaphtalene 8-sulfonate, CFDA-AM:6-carboxyfluorescein diacetate acetoxymethyl ester, EB:ethidium bromide.
A: differential contrast image, B: fluorescence image (ANS), C: fluorescence image (CFDA-AM), D: fluorescence image (EB).
Objects marked by → seem to be detritus because they are not detectable in B and C. On the other hand, objects marked by ★ can be recognized as microorganisms because they are fluorescent in B, C and D. Actually, they are spores of the cellular slime mold.
scale:20 μm

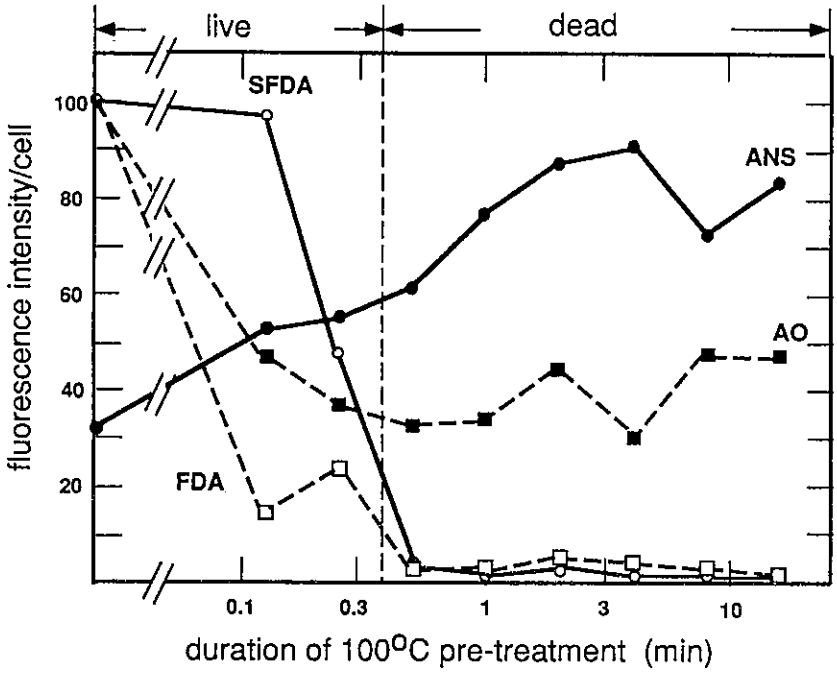

Fig.3. Discrimination of live and dead by fluorescent probes (13)

Cultured *Escherichia. coli* was heat treated (100℃) for indicated times and stained with the fluorescent probes. Aliquots were subjected to plate culture and remainders were subjected to fluorescence measurements.
AO:acridine orange, ANS:1-anilinonaphtalene 8-sulfonate, FDA:fluorescein diacetate, SFDA: 5-(and -6)-sulfofluorescein diacetate.

Note that SFDA is sensitve to proliferation capability, while ANS is suitable for whole cell (including dead and non-proliferative) detection.

E. When soil specimens were measured, the fluorescence microscopic technique yielded 50- 100 fold larger cell density than the plate counting technique. This suggests that the present technique detects so called viable but non-culturable cells (16,17), and also suggests that in natural environments, majority of microorganisms are difficult to culture.

F. Vertical distribution of microorganism of soil microorganisms from Mt. Shigayama showed that, at surface, cell density was small and maximum was shown

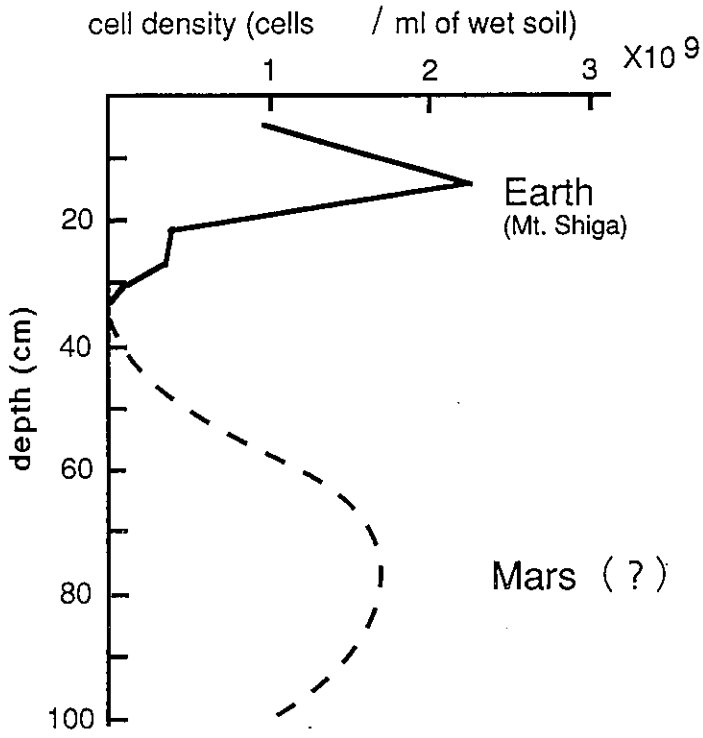

Fig.4. Vertical distribution of microorganisms in soil (modified from 13)

Soil was sampled from different depth at Mt.Shiga (Nagano) and stained with SFDA. Cell density was determined from fluorescence images of SFDA.

Note that maximum cell density was obtained 15 cm below surface even on Earth. Since surface condition is much harsh on Mars, putative Mars life will be found at much deeper depth.

below 15 cm from surface (Fig.4). At Mars, it is known that the surface environment is so harsh for life to survive due to radiation and superoxides. We must explore deep in soil.

G. Microorganisms in the simulated Mars atmosphere and irradiated by protons could be detected by SFDA or ANS even after they lost reproductivity. Even if expected Mars life lost reproductivity, the fluororescent probes shown above may detect them (Fig.5).

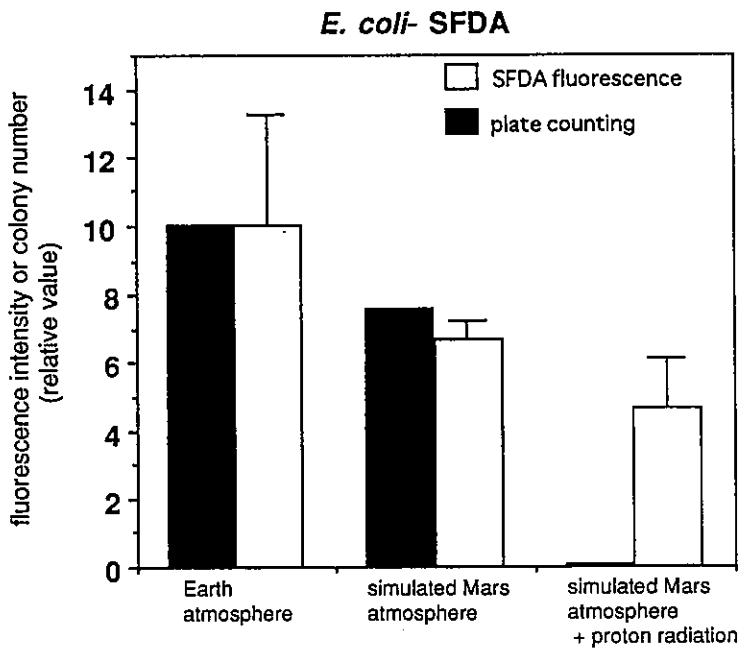

Fig.5. Effect of simulated Mars atmosphere and proton irradiation on the survival of *Escherichia coli.*
Comparison of fluorescence microscopic and plate counting methods.

Escherichia.coli cells were kept in usual Earth atmosphere and simulated Mars atmosphere. Aliquots of the latter was subjected to proton radiation with van de Graaf type proton accelerator (1M eV, 250 year accumulated amount at Mars surface). After each treatment, they were subjected to the plate counting and the fluorescence microscopic analysis (using SFDA) for counting viable fraction.

Note that cells kept in simulated Mars atmosphere and irradiated with proton show no colony in the plate counting analysis. On the other hand, by the SFDA fluorescence analysis, the cells show 45% fluorescence intensity of the control (Earth atmosphere) . This means that these cells still contain esterases and other molecules to maintain the cleaved products of SFDA. These cells may be viable but not reproductive or just after death.

H. Some pre-biotic cell {proteinoid: cell like aggregates obtained by heat treating amino acids (18) } could be detected by SFDA or ANS . If putative extraterrestrial life is still under evolution to complete cells or they stopped evolution at pre-biotic stages , there is a good chance that the present method detects them.

I. We are now developing a full automatic and compact system targeting field and Mars exploration (Fig.6).

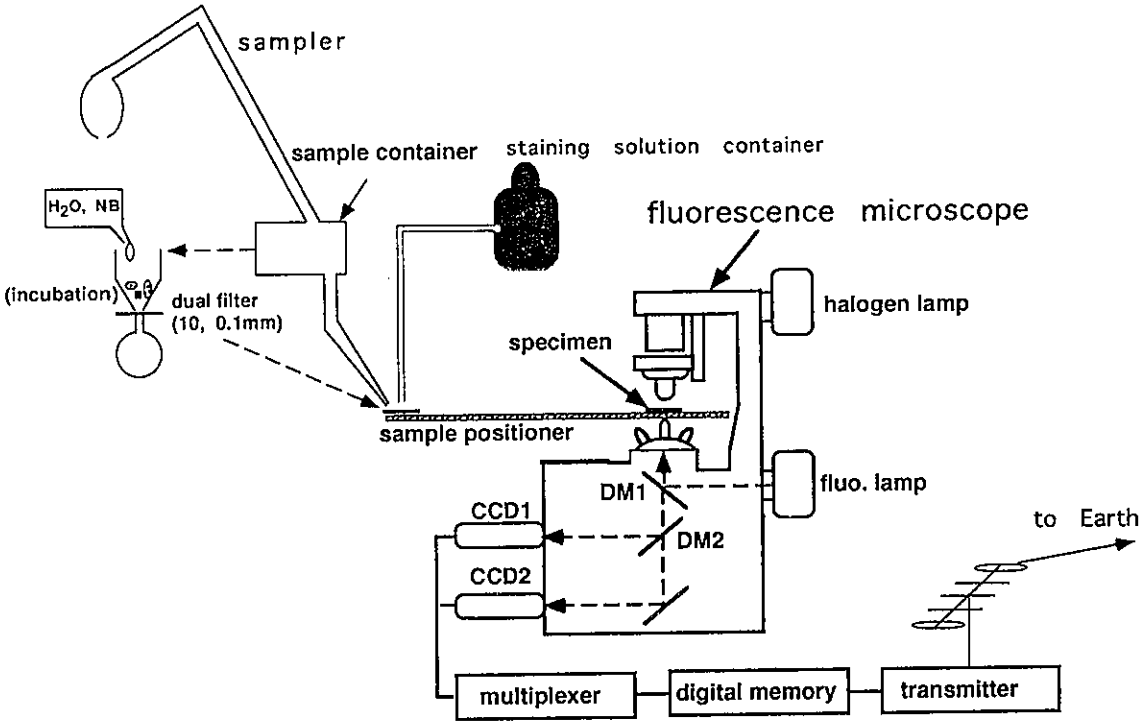

Fig.6. Detection system of Mars Life

Specimen sampled by boring machine is kept in the sample container, and aliquots are put on the sample positioner and set at just-focus position. Images are acquisitted by the (cooled) CCD and transmitted to Earth. Sampling locations are determined from Earth after judging the obtained images. In this figure, 2 cameras are
equipped. However, as explained in Fig.1, loading of 3 cameras is ideal corresponding to 3 major characteristics of Life.

It can be concluded that the fluorescence technique is complimentary to the conventional plate counting method and is one of the most promising for the exploration of unidentified and sparsely distributed microorganisms.

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Acknowledgements

The authers will represent thier gratitude to Dr. H. Yanagawa and Miss Y. Ogawa (Mitsubishi-Kasei Institute of Life Scicences) for providing proteinoids.

生物学的情報の起源

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要旨

情報の発生、進化、蓄積が可能な情報システム(IS)の特性について考察した。アイゲンのいう様々なタイプの「ハイパーサイクル」を含む前生物的なISの生成について議論した。ハイパーサイクルの目的は、自分自身の情報を保持しようとすることと定義する。この目的に沿った情報の質は、その選択の過程において、ゼロから極大値もしくは極小値へと徐々に増加もしくは減少することが示された。また、高品質な有益な情報質が、突如として現れてくることはありえないが、徐々には現れてくる可能性は高いことが示された。

Viva Origino 24(1996)305−320

ORIGIN OF BIOLOGICAL INFORMATION

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(Received June 20, 1995, Accepted August 17, 1996)

Abstract

Properties of Informational System (IS) where the generation of information. its evolution and storage are possible. are considered. The formation of pre–biological IS containing various type hyper–cycles (in the Eigen sense) is discussed. It is proposed to define the aim as the aspiration to keep own information. It is shown that the quality of information according to this aim increases (or decreases) gradually from zero up to maximal (or minimal) value during the process of choice. It is shown that the probability of gradual appearance of high quantity of valuable information is not small. whereas its abrupt appearance is practically improbable.

1 Introduction

There are three stages of life origin:

1. Formation of the basic biochemical substances (nucleotids. amino–acids. and so on) in nonequilibrium conditions (in volcano. lighting. etc.).This problem is in principle solved now [1. 2. 3].

2. Formation of polymers (DNA. RNA. peptides. lipids) and their *self–organization* [3]. i.e. formation of coacervates [4. 5]. microspheres [6. 7] or marigranules [8. 9]. It has been shown that condensation of nucleotides (formation of polynucleotides : DNA. RNA)

and amino-acids (formation of polypeptides) is possible under certain conditions. The polymer sequences occur to be accidental in this case, and polypeptides have no specific biochemical functions. However in this very case polymers are able to self-organize themselves.

3. Generation of biological information.[10] i. e.:
   (a) formation of non-accidental polymers possessing the specific biological functions.[11, 12]
   (b) origin of unique code [11, 12, 13, 14],
   (c) origin of biological asymmetry [14, 15].

The last problem is of interest and actively investigated now . Note that the problem of valuable information origin is more broad: it includes some problems of origin of languages and alphabets [14], generation of scientific and technical ideas [16, 17, 18, 19] (so called creation problem), etc. We consider here this very problem.

Before the discussion of biological aspects some short excursion to the theory of information is reasonable.

2 Excursion to the Information Theory and Definitions of the Basic Concepts

2.1 Two Concepts of Information

There are two concepts of information:

1. Micro-information — is a choice of one version from N possible ones.
2. Macro-information — is a stored choice of one version from N possible ones [20].

The difference between micro and macro information is in the condition of remembering which is very important.

The example of micro-information is the choice of positions and velocities of molecules of an ideal gas. Accordingly to the hergodic principle this choice can not be remembered; this condition is necessary for the formulation of concept of physical entropy. Well known statement that

the entropy is nego-information is valid for micro-information only, it is based on the condition of "forgetting of the micro-states" in the ergodic systems.

The example of macro-information is the choice of some definite consequence of different nucleotides in DNA. This choice can not be changed during life time of of macro molecule, i.e. it is memorized for this time. The remembering is a macro process, it can proceed in macro-systems (at least in macro molecules). The remembering is a dissipative process, it is accompanied by the entropy production (its quantity being much more than that of the macro-information appeared) [16, 17]. Thus the macro-information has no direct connection with physical entropy. The concept of memory requires to determine the time of remembering (a eternal memory exists in necrologies only, while in real nature memory time is always limited). If this time is much more than relaxation time of a thermal fluctuation (which is about 10^{-13} s under room temperature) then the information is macro-information with respect to the micro-one. If this time is much less than the characteristic time of the process in question then the information is to be treated as micro- (or semi-micro) information, but not as macro one. We point out these questions because there are important but not widely discussed now.

In applications of the concept of information one always is dealing with macro-information; in the following we shall use the term information as the macro-information (if it is not especially mentioned).

2.2 Some Properties of Information

2.1.1. The quantity of information is equal to :

$$I = -\sum_{j}^{N} p_j \, log(p_j) \qquad (1)$$

where p_j being *a priori* probability to chose the j-th version from N possible ones. If these probabilities are equal to each others and to $1/N$, then the quantity information is $log_2 N$.

2.1.2. Reception of Information is a predetermined choice provided by some external forces (or signals) and/or proceeding evolution of the system.

2.1.3. Origin of Information is an accidental choice.

2.1.4. Value (or *quality*) of Information depends on the aim. According to Bongard [21] and Kharkevich [22], this value is equal to :

$$V_i = log_2 \frac{p_{fin}}{p_{in}} \tag{2}$$

where the p_{in} is the *apriori* probability (before the information is obtained); p_{fin} is the probability to achieve the aim (after the information of the i–th type is obtained).

2.1.5. Informational System (IS) is that is able to make a choice (due to accident and/or external signal) and remember it for long enough time. Principal properties of the IS are the following:

a) IS should be multi–stationary one;

b) IS should be dissipative one;

c) IS should have a quasi–chaotic stage in the process of its working (it is necessary for accidental generation of information);

d) IS should be able to rmember a chosen version for a long enough time.

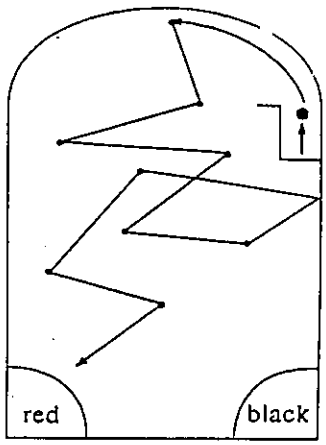

Figure 1: Scheme of the chinese roulette, • — the boll, the points correspond to the nailes, broken line is boll's trajectory. (a) Initial stage; (b) Chaotic stage; (c) Final dynamical stage.

The example of IS is the Monte–Carlo (or chinese) roulette: (see Fig. 1) it is multi-stationary system (the number of stationary states is equal to the number of holes at the ring); it is dissipative — the ball falling into the hole looses its energy, that provides the storage of the choice for a definite time. There are three stages of the process : initial stage (croupier pushes the ball), chaotic motion of the ball between the nails (which provides an accidental choice of the hole), and the final stage (when the ball almost occupies some definite hole). The later is dynamic one. The result of the process at this stage is predictable.

Note that IS can contain (as well as it can not) information. In any case this IS is to exist before generation (or obtaining) of this information. E.g., the roulette is to exist before the game, the last is the process of information generation.

3 Information System in Pre-biotic Period

We suppose that the medium at this stage contains nucleotides, amino-acids polynucleotides, polypeptides and lipids. These substances formed the coacervates (or microspheres, or marigranules) where concentration of the above mentioned ingredient was high enough for formation of the complexes of polynucleotides with peptides.

This system satisfies the conditions (a), (b), (c) which are necessary for a system to be IS. The condition for a long time remembering requires special discussion.

The point is that the choice of the sequence of any polynucleotid is stored for its life time only. This time is rather short as compared to the evolution time. For a long time remembering a complementary auto reproduction of DNA is necessary. The last is really possible in the case of participation of protein with specific functions, i.e. the protein-replicase, while its synthesis requires participating of DNA. Thus we are dealing with the closed circle: the protein-replicase promotes complementary reproduction of DNA, and DNA should promote synthesis of the protein-replicase. This problem has been considered by M.Eigen [23, 24] and denoted as the *hypercycle*. The pair DNA-protein-replicase is the simplest example of hypercycle.

In modern biosystems this closed circle is provided by a system of the protein biosynthesis. The last contains the t-RNA, the ribosome and a set of adapters (this role is played by the amino-acilyadenilate synthetases). A modern biosynthetic system contains a great quantity of valuable information (including the information on the biological code), that has been estimated in [12] as $I = 3300$ bit.

This system was absent at prebiotic time. The probability of spontaneous appearance of such a system by one step is extemaly small :

$$W \sim 2^{-3300} = 10^{-1000} .$$

The attempts to imagine some more simple system of biosynthesis (including however the process of coding which contains a great quantity of valuable information) result in the same problem: the probability appears to be too small.

Another approach to this problem has been suggested in our papers [11, 12, 14]. We have considered the mechanism of the protein-replicase

formation in the presence of DNA but without the biosyntetic apparatus (i.e. without the coding and biological information). This mechanism is based on the principle of heterogeneous catalysis where DNA acts as matrixes. Amino-acids are absorbed on these matrix and their further condensation leads to formation of the protein in cover form, with its internal surface being the molding from external surface of DNA. The cover-type protein is capable:

1. to act as a replicase of the DNA used for its formation under periodical conditions (i.e. change of temperature, pH, etc);

2. to work as an inhibitor for DNA of some different sequence.

Possible mechanism of formation and working of cover-type protein is discussed in more detail in the appendix.

The complex containing DNA and corresponding cover-type protein-replicase is a hypercycle of the i-th type capable for auto reproduction. The index i corresponds to the nucleotide sequence in DNA; it is equal to: $i = 1, 2, ..., N$, with $N = 4^n$ and n being the number of nucleotides in this DNA. System containing a set of different hypercycles is an Informational System (IS) capable to make a choice (i.e. to choose the one type of hypercycle from N possible ones) and remember it. Note that this IS does not contain yet an information because possible versions of hypercycles are presented and none has been chosen. The surviving of one concrete type of hypercycle just means the choice of the code version.

The process of choosing depends on concentration and interaction of different type hypercycles. To consider this process it is convenient to use a mathematical model.

4 Mathematical Model

Let us consider the symmetrical model for interaction of hypercycles with equal abilities for surviving. This model in dimensionless form is the following [11, 14, 19] :

$$\frac{\partial u_i}{\partial t} = u_i - \sum_{j \neq i} u_j u_i - a u_i^2 + \Delta u_i, \quad i = 1, 2, ..., N, \tag{3}$$

where u_i is the concentration of the i-th type hypercycle, a - is a model parameter ($a < 1$). The first term describes an auto reproduction: the

second term corresponds to the antagonistic interaction between hypercycles of different types; the third term reflects the effect of tightness. The condition $a < 1$ means that antagonism between similar hypercycles is less than that of different ones. The last term describes the space spreading of hypercycles (Δ being the Laplace operator).

This system (3) possesses the following properties:

1. there are N "pure" homogeneous stable states where $u_i = 1/a = Const$; $u_{i \neq j} = 0$.

2. there are two symmetrical homogeneous unstable states, the first one being of the saddle type:

$$u_1 = u_2 = ... = u_N = 1/(N - 1 + a)$$

while the second being of node type: $u_1 = u_2 = .. = u_N = 0$.

3. the evolution of the system, starting from the saddle type unstable state, contains three stages (shown in Fig. 2, *a*)–*c*) for the case of two-dimensional space and $N = 3$).

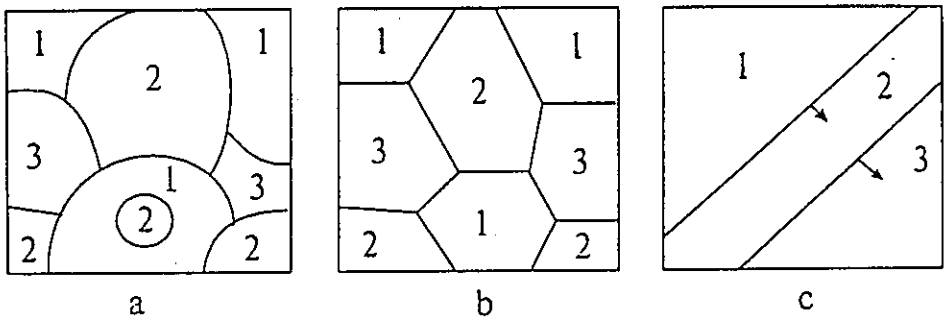

Figure 2: Three stages of the development of system (3). (a) Initial stage; (b) Chaotic stage (parquette); (c) Final dynamical stage.

I Formation of "pure" clusters (or "bubbles") where $u_i \gg u_{j \neq i}$, divided by boundaries. This stage is short.

II Formation of "pure" clusters with direct boundaries ("parquet") and slow motion of these boundaries so that large clusters force out small ones.

III Formation of the pure homogeneous stable state. This process has quasi-chaotic character at the two first stages. It means that the result is unpredictable (i.e. accidental one): small external fluctuations can change the result drastically. At the final III-th stage this process has dynamic character and the result is quite predictable. Thus the choice of the version (i.e. the type of hypercycle) occurs at the moment of transition from the parquet stage to the final one. At the final stage this choice is only fixed and thus becomes to be the remembered one. This process is analogous to the ball motion in the roulette.

Let us discuss informational aspects of the process in question. This process describes the choosing of the only version among N equal ones in the whole system. This process is accidental because of instability of initial state as well as first two stages. The situation corresponds to Orwell's thesis: "All animals are equal, but some of then are more equal than others". The choice appears to be memorized because the final state is stable. Thus the process describes generation of information.

The choice of the version of i-th type hypercycle takes place at the moment of its appearance. The choice is an accidental one under the spontaneous condensation of nucleotides; the choice is receipted from parents at the stage of auto reproduction of the hypercycle. At the first two stages the version choosed by every hypercycle is not remembered, so every hypercycle, starting from the moment of its origin, contains information, however this information is micro (or semi-micro) one with respect to the evolution time scale. At the final stage the version chosen by the population of hypercycles which wone becomes to be common for the whole system and remembered one.

The value of the chosen information depends on the aim and on the stage of process. In the frame of work of the model (3) the aim of every hypercycle is to survive. One can say in more general form that the aim of the i-th type hypercycle population is the storage of own information. The aim of the whole system is absent up to the final stage. When the system acquires the information, it tends to keep this information, i.e. the aim of the whole system is also the storage of its information.

Basing on this consideration and the expression (2) one can estimate the value of information depending on time. The value of any information is zero at the initial moment because an a priori probability to achieve the aim p_{in} is equal to final one (independently on the index i). At the final moment, when the i-th type hypercycle has wone the information of the i-th type becomes valuable and positive while the information of

any other type ($j \neq i$) becomes to be negative. Thus the value of information increases (or decreases) gradually during the process of choosing. The value of information changes drastically at the moment of transition from the parquet to the final stage. The gradual increase of the value of information is important in connection with the problem of small probability. Indeed, any information is not valuable at the moment of its origin because the probability of this act is not too small: the probability of surviving of any type of hypercycle is equal to unity. During this process the information of the conqueror becomes valuable, but the probability of its origin is not too small again.

Let us illustrate this result using the Monte–Carlo roulette. Let somebody whose aim is to win has put his money on the "red" (it is the act of generation of information). The value of this information is zero at the initial moment because the probability to win (as well as to lose) before the act of generation of information and after it are equal to each other. At the final moment, when the ball is in some definite hole, the information is valuable and positive (if the ball is really in the red hole) or negative (if not). The player can put his money in some intermediate moment before the croupier said the words " the game is done". An experienced croupier use to say these words at the transition time interval. If the croupier is lazy or crazy he can say the crucial words after the transitional moment, then the player can choose the right version and win the money with the probability more than 1/2. Thus the transition interval is the optimal one for the generation of the valuable information, with its value is not zero at the generation moment.

The hypercycles in the considered above model can not change their information; it could be possible in more complicated model able to describe the process of creation.

5 Conclusion

I Let us formulate shortly the main results.

1. The process of valuable information origin is divided on two stages:
   - origin of the IS
   - gradual increase of the value of information during the process of choosing.

2. The prebiotic TS should contain an auto reproductive complex DNA–protein-replicase (i.e. the hypercycle in Eigen's sense). Possible mechanism of the protein–replicase with participation of DNA but without the coding process was considered.

3. The aim of the i–th type hypercycle has been formulated as a tendension to keep its own information.

4. The probability of the process of gradual origin of high quantity of information has been estimated to be not too small, whereas abrupt origin is practically improbable.

II The process in question is convergent one because it is accompanied by formation of the unique specie of the primary organisms. Further divergent stage (formation of variety of species) requires for special consideration [14, 25]. Necessary conditions for the divergent stage are the following :

1. formation of polyploids containing the chain of many hypercycles of the same type;

2. mutation of the DNA at some points of the chain, whereas the others are to remain unchanged [14, 25]. The last is necessary for observance of succession in evolution. The repeated information in the chain can be considered as a neutral information (in the Kimura's sense [26, 27]) what is needed for the divergent evolution.

III We have not touch the problem of adapters formation (i.e precoursors of amino acyladenilate synthetases) which had been considered in [11, 14]. In principle this process in primary hypercycles can proceed by the molding mechanism, i.e. without coding system.

Appendix

Formation of protein in presense of DNA can proceed by the following way:

The fitrst stage is the adsorbtion of activated aminoacidts on the DNA. It sould proceed in soft conditions (temperature,pH, etc.,where the charges of phosphate groups are compensated). Adsorbtion provided by two types of interactions: (a) specific bonds of some am.–acides with

defined two or three nucleotides (i.e. codons) [13, 28] and (b) nonspecific Van der Vaals bonds of am.-acides with the surfase of DNA. The absorbtion of different am.-acides proceeds independently and thus complementarity of adsorbent to adsorbate should be maximal one.

The second stage is the condensation of the adsorbed am.-acides in relatively hard conditions. The sequence of the stages is important because molecules of DNA, which are not protected by adsorbed am.-acides,would be hydrolysed in conditions of condensation.

We denote the complex which is formed in this way by hypercycle of i-th type, where the index "i" corresponds to the sequence of the used DNA. We denote the cover type proteine in this complex and the corresponding DNA by "own" to each other. The binding of such protein with some another DNA (not " own") is less then that with the "own" one because the specific interactions and Van der Vaals ones prevent each other in this case. The correspondence between the codons of DNA and the ajoining to them am.-acides in the hypercycle of the i-type forms the some version of code.These versions are different for different types of hypercycles.

Thus, the choice of the type of hypercycle is the choise of the version of the code.

The properties of the cover type proteine as enzyme should be the following:

1. It should work as an protector of DNA at stationar conditions.

2. It should work as initiator of the replication (i.e. as an replicase) at the periodic condition with respect to the " own " DNA. Supposed mechanism of initiation is the following (it is based on the protein-engine concept [29]): change of the conditions leads to a change of the form of proteine (i.e. to the conformational transition) so the throat of the cover becomes more broad. It leads to consequenses of two kinds depending on the stregth of the bonds: if the strength of the bonds of the proteine with the DNAis more then that of hydrogen bindings between the nucleotides, the later appear to be broken that promotes to the initiation of the replication. In the opposite case the effect of initiation should be absent.

3. It should work as isosteric inhibitor of replication with respect to " not-own " DNA preventing of formation of the productive complex.

Interaction of the hypercycles of different types (i–th and j–th) means that the exchange of its "covers" can proceed in the mixture. It leads to the inhibition of the reproduction of both populations. It is the cause of antagonistic interactions.

Acknowlegement

We are thankfull very much to professors K.Gladilin, V.Otroshchenko, S.Fox, A.T.Pol and A.Pappelis for fruitfull discussions.

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Viva Origino　24巻4号

1996年12月20日　印刷
1996年12月25日　発行

編集者　〒590　大阪府泉南郡熊取町野田1010　京都大学原子炉実験所内
生命の起原および進化学会編集部
印刷者　〒594　大阪府和泉市池上町460－33
和泉出版印刷㈱　TEL 0725-45-2360　FAX 0725-45-6398
発行者及び出版者　〒560　豊中市待兼山町1-16　大阪大学大学院理学研究科生物科学専攻内
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