NOT «ORIGINS OF LIFE» BUT «EVOLUTION IN MICROBES»

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> *The present is the key to the past.*
>
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INTRODUCTION

Our present lack of knowledge of other life forms makes it difficult to hypothesize about, and even harder to design experiments to reproduce the conditions and results of the first emergence of life in the universe. Even if we restrict our scope to the Earth, limiting our research possibilities to carbon-based life forms and Earth-like planetary conditions, the gap between prokaryotic cellular organization and the earliest possible self-maintaining systems is so large that we currently cannot extrapolate backwards to make a logical bridge between them.

Only living beings have shown the ability to retain and utilize information during the course of Earth's history. The accumulation of information is a property of life. Two essential features of the information storage systems of organisms are the ability to replicate messages and the capacity to duplicate the entire set of molecules and structures produced by these messages, i.e., the capacity to reproduce cells.

Elso Barghoorn, the father of pre-Phanerozoic paleobiology, was fond of saying that he did not study the origin of life, an idea shrouded in mystery, but rather that he was interested in the antiquity of life (Margulis and Stolz, 1984). Current scientific attempts to resolve the mystery of the origin of life usually deal with tentative extrapolations from the possibilities inherent in certain chemical reactions. Extrapolations or logical inferences from «prebiotic chemical systems» have not yet explained what actually occurred on the early Earth. Nor is there any body of current scientific knowledge that allows us to predict the outcome of attempts to produce an autopoietic system in the laboratory.

BIOCHEMICAL VS. CELLULAR SYSTEMS APPROACH

We can divide the scientists studying the origin of life into two groups: those attempting to mimic the simplest structures and functions with autopoietic capacity (a biochemical system approach), and those trying to dissect the essential structures and functions of living cells to analyze the minimal conditions for life (a cellular system approach).

The present gap between the experimental gains of the former and the maximum possible simplification of the latter is enormous. It is large enough to conclude that we are still very far from knowing how life began its history on Earth.

The main purpose of this article is to epistemologically reduce that gap in the form of an inquiry into the essential mechanisms and structures of the earliest living forms. What were the minimal requirements for the multiplication and growth of the earliest beings in their ancient habitats? We proceed as follows: (1) We distinguish many features of cells, both their components and operations, that evolved subsequent to the origin of life itself. Such components and capabilities have been characteristic of life since the Archean Aeon but they were not essential for the reproduction of the earliest cells. (2) We attempt to reduce to a minimum those common imperative operations of the simplest (prokaryotic) cells, the bacteria. We propose that the complexity of metabolism is a product of bacterial evolution. And (3) we emphasize the great importance of the environment for Archean evolution of bacteria. Only when we consider the subtleties and gradients of the environmental parameters which surround a given bacterial population is it possible to understand the changes in number and in physiological state of the cells comprising that population. Only through interaction of the different populations that form bacterial communities did early life maintain itself by cycling the chemical elements and using external energy sources. Throughout the history of life and since its origin, different bacterial populations were provided with environmental sources of energy, carbon, other elements and reducing power, eventually organizing themselves into microbial ecosystems.

We present, in approximate chronological order, increasingly organized steps in the evolution of life. We begin with the first autopoietic systems, assuming they had the structure and metabolism of simple fermentative obligately anaerobic bacterial cells. We proceed to the essential structures and functions of the first eukaryotic cell, the prerequisite for the explosive Phanerozoic radiation of animals and plants. We see that many of the characteristics thought to be the results of animal and plant evolution had already appeared in microorganisms by the beginning of the Cambrian Era.

AUTOPOIESY

The problem of the origins of life has become the problem of autopoiesy, that is: how did the minimal autopoietic entity come into being? We now believe that the minimal autopoietic entity is a bacterial cell, or, because, it is the nature of cells to multiply, a population of bacterial cells. But what is an autopoietic system, what is autopoietic life?

Autopoietic entities are self-making and self-maintaining. They have boundaries (cell membranes, cuticles, skin) that clearly mark their relation with the outside. They transform matter and energy, add and take away bits of themselves and maintain their identity. Autopoietic entities respond to external conditions by self-preserving activities. They heal their mem-

branes, rewax their cuticles, grow back and cover lesions in their skin. Within a range of limits of nutrients, light, temperature and other external changing factors, autopoietic entities are recognizably themselves.

In 1973, Francisco Varela and Humberto Maturana developed their concept of autopoiesy, recognizing that it fits with what we expect of life (Maturana and Varela, 1980). «Reproduction and mutation and the capacity to transmit mutation» alone, although popular, is not an adequate definition of life, since many sterile animals and old women who will not reproduce and mutate are certainly still alive. Indeed, they are autopoietic entities that insure own persistence.

Viruses, in the absence of their host cells, are not autopoietic entities. They lack dynamic borders maintained by active interchange of parts. They respond passively to their environment and its change. Viruses only behave in an autopoietic way when they are inside of an autopoietic host cell. Hence the minimal autopoietic entity is still a collection of prokaryotic cells, and the origin of life is the problem of the origin of a set of metabolizing membrane-bound spherical entities of less than a micrometer in diameter (namely, a population of bacterial cells).

SEMES

The concept of «seme» and its implications

When the living world was divided into the two kingdoms, Animals and Plants, the problem of the origins of life was conceived of as the problem of the origins of plants. Since it was patently obvious that animals cannot exist without the benefit of food generated by photosynthesis, and since in the dichotomizing limited mind of man, Animal or Plant were considered the only possible forms of life, the «origin of life problem» was the same as «the origin of the primitive plant» problem.

We know now that the «origin of plants» is an enormous complexity of problems that include events at many different levels at very different times during the history of life on Earth. For example, the «origin of plants» problem includes the problems of the origins of prokaryotes (bacterial cells), of photosynthesis, of eukaryotic cells, of symbiotic cyanobacteria that became chloroplasts, of cell motility, of mitosis and sexual reproduction, and other problems. Now that the living world is much more appropriately divided into prokaryotes and eukaryotes we begin to define the «origins of life problem» as the problem of the origins of the first spherical heterotrophic fermenting anaerobic bacteria. Yet the old errors continue as so many authors consider the origins of life a single event, trying to explain too much at once. In this discussion, we hope to distinguish the origin of life problem from a whole series of other issues, often confused, that clearly belong to the domain of the evolution of microbial life.

The very first bacteria-like cells probably were much less complex than even the simplest extant life. A recent interesting idea is that the first cells were RNA-cells, composed only of RNA and protein, that is, of membrane-associated catalytic and replicating RNA (Lazcano et al. 1987). If this view is correct even DNA metabolism was a product of microbial evolution. In any case, following the original analysis of Morowitz (1967), we can recognize 0.5 μm diameter wall-less spheres as common ancestors of all life on Earth. From such autopoietic entities and their DNA, the universal features of all present-day autopoietic entities evolved: messenger RNA, and its triplet code, acylating enzymes, lipoprotein membranes, and many features (semes) which we attempt to list below.

Semes are useful units to deal with in a discussion of evolution such as this one. They are features of organisms that have clearly identifiable selective advantages. They are the characteristcs with which evolutionists traditionally deal: fruits, eyes, uteri, oxygen respiration, swim bladders, nitrogen fixation, etc. Many genes are required to make any seme. Not to repeat what has been written elsewhere, we simply outline, in rough order of appearance, those new semes, that is «neosemes», that clearly evolved inside microbes and therefore are not intrinsic to the origin of life problem.

A seme can be thought of as an inherited trait or feature, the product of many genes, of clear selective advantage to the organisms that carry it. The appearance of new semes depends on the net accumulation of genetic material. Among extant organisms we know that genetic material, in the form of lengths of DNA, can be acquired by many mechanisms including replication of genomes in the absence of cell division, gene duplication, phage or plasmid transfer, polyploidization, organellar replication and even hereditary symbiosis. Of course genes can be modified and lost by complimentary mechanisms, but the net loss or alteration by mutation of genetic material is of less consequence to the appearance of neosemes than the net gain.

By the distribution of and variation in semes it is possible to reconstruct the group of organisms in which they first appeared. Using this evolutionary practice we summarize here distinct stages in the origin and evolution of the first, necessarily microbial, autopoietic systems. Our summary is in the form of a list of semes, in roughly what we think may have been a chronological order, beginning with the earliest semes to have appeared.

List of semes appearing before or during the emergence of the first prokaryotes ancestral to subsequent life

1) RNA polymerase and other proteins, as well as the accompanying nucleoside triphosphate (ATP, GTP) metabolisms, are the *sine qua non* of the Earth's autopoietic system, probably «RNA-cells» (Lazcano, 1987; Lazcano *et al.*, 1987).

2) Due to the segregation of the autopoietic system from the environment, lipid metabolism involved in the production of cell membrane must have also occurred in these earliest common ancestors. The central role and universal distribution of acetate suggests that from the beginning, all life on Earth had a capacity for acetate metabolism.

3) Ribosome-mediated protein synthesis evolved inside cells, according to the analysis of Lazcano (1987).

4) DNA-mediated messenger RNA, deoxyribose metabolism and other steps in DNA synthesis also evolved in RNA-cells (Lazcano, 1987).

5) DNA polymerase, ligase, restriction endonucleases and editing and repair systems in general, because apparently they are universally distributed, evolved in the common ancestors of all life (Doolittle *et al.*, 1986).

6) Ion channels for sodium, potassium and calcium, the basis of internal ion control, also distributed ubiquitously in living organisms, must have been present in our common ancestors. Since ion pumping forms an essential component of autopoiesy it probably evolved prior to or during the emergence of the first common prikaryotic ancestors. Membrane control of ions is an intrinsic feature of the self-maintaining system of any cell (Harold, 1986),

List of semes appearing after the first common prokaryote

1) The evolution of transcription, the process that copies lengths of RNA from DNA in a precise order given by the rules of base pairing probably evolved in bacteria (Zillig *et al.*, 1985). Studies of the enzymes involved in RNA synthesis suggest that there are at least two different forms of RNA synthesis in bacteria: archaebacterial and eubacterial. The transcriptional apparatus in these groups of bacteria apparently diverged early in the history of life on Earth. The presence of intervening sequences in these groups of bacteria, e.g., in archaebacterial DNA, suggests that eukaryotes merely elaborated on a genetic organization already present in their prokaryotic ancestors.

2) DNA splicing and ligating enzymes, topoisomerases and other requisites for DNA repair and combination presumably evolved in common prokaryotic ancestors in response to ultraviolet light and other mutagenic threats.

3) Murein, pseudomurein and peptidoglycan cell walls clearly evolved in different ways in different lineages of prokaryotes (Kandler, 1985). Because cell walls differ in composition and construction and some bacteria are without them, it is clear that walls are not a necessary component of all autopoietic systems.

4) Fermentation of small organic molecules (i.e., the metabolism characteristic of bacteria entirely intolerant of molecular oxygen) is probably the kind of metabolism that requires the smallest quantity of genetic material (i.e., fewer enzymes) to manage it.

5) Desiccation resistant spores clearly evolved in bacteria that ferment small carbon compounds in absence of oxygen (such as *Clostridium*). An ancient origin of these useful structures is likely.

6) Rotary motors, the cell wall «wheels» of flagellated bacteria, are present in a number of types of bacteria that in the absence of oxygen ferment carbon compounds, suggesting the early appearance of this most useful seme.

7) Flagellated bacteria are often chemotactic, displaying sensitivities to (moving toward and away from) various organic compounds found in their surroundings. The appearance of locomotory behaviour sensitive to chemical stimuli probably first occurred in flagellated bacteria where it is still found today. Ancestors to such flagellated bacteria also evolved before free oxygen appeared in the Earth's atmosphere.

8) The first production of hard minerals inside the cytoplasm of cells (biomineralization) is associated with microaerophilic, motile, magnetotactic bacteria. Biomineralization too probably had an early origin in bacteria (Westbroek and de Jong, 1983).

9) Methanogensis, the production of gaseous methane from CO_2 and H_2, occurs in archaebacteria. This sort of metabolism which requires the absence of oxygen presumably is the earliest form of chemoautotrophy.

10) Isopentyl pyrophosphate metabolism, which is nearly universally distributed, is an absolute prerequisite for chlorophyll reaction center photosynthesis, and must therefore have preceded any photosynthetic system.

11) Photoheterotrophy. Light-sensitive membranes (rhodopsin) and photophosphorylation of ATP presumably is far less complex than photoautotrophy. Since it therefore requires a less extensive genetic system for its administration it probably evolved in bacteria prior to photoautotrophy. Notwithstanding, a continuous supply of organics would have been necessary even in Earth's earliest ecosystems, and this would have been produced by incorporation of light energy, i.e., primary production.

12) Anoxygenic photosynthesis evolved

in several lineages of bacteria which thus must have had light-sensitive membranes and the chlorophylls, carotenoids and porphyrins which comprise them.

13) Since sulfate reduction (i.e., sulfate respiration) is even today limited to anaerobic bacteria, clearly it evolved in this group of organisms, and probably from a simplification of the electron chain of anoxygenic photosynthesis (Trüper, 1982).

14) Chemolithotrophy based on sulfide, ammonia, hydrogen and methane oxidation are bacterial modes of metabolism characteristic of different lineages of bacteria that require gaseous oxygen. They obviously evolved in bacteria since they are not found in other organisms.

15) Differentiation, that is, linear development based on multicellularity such as that present in heterocyst-forming cyanobacteria, evolved in prokaryotes, where it still can be found.

16) Oxygenic photosynthesis evolved in the anaerobic-anoxygenic ancestors to cyanobacteria and *Prochloron*.

17) Predation, the attachment, penetration and killing of one type of bacterium by another, also evolved in anaerobic bacteria, as in the cases of *Vampirococcus* and *Daptobacter* (Guerrero *et al.*, 1986, 1987).

18) Nitrate respiration, present in facultative anaerobic bacteria and unknown in eukaryotes, evolved in obligately anaerobic bacteria.

19) Aerobic (free oxygen) respiration probably evolved in those bacteria that already contained cytochrome chains for the respiration of nitrate.

20) Myxobacterial and actinobacterial multicellularity involving the filamentous habit, exospore and cyst formation, evolved in various lineages of bacteria.

21) DNA-coated protein, HT-A histone-like protein and acid tolerance evolved in the ancestors to *Thermoplasma*, probably an archaebacterium (Searcy and Delange, 1980).

22) Since methylotrophs are capable of steroidogenesis, this process too evolved in bacteria, albeit an extremely limited group of bacteria.

23) Stable associations of prokaryotes for the origins of organelles are known in modern bacteria (e.g., «Pelochromatium») and can be considered preadaptations to the origins of those intracellular organelles of eukaryotes that demonstrate genetic continuity.

24) Internal membranes for DNA segregation (including nuclear membrane and outer organellar membranes) probably evolved concurrently with intracellular associations of bacteria.

This list, clearly oversimplified, can be augmented by a second list (see next section) derived from the appearance in the Proterozoic Aeon of the eukaryotic level of cell organization.

List of semes appearing after the first eukaryotes

1) Since intracellular motility is limited to eukaryotes, it probably evolved in the earliest eukaryotic forms. One of the earliest intracellular motility systems is that of undulipodia (e.g., cilia) which may have evolved by symbiotic acquisition of spirochetes (Margulis and Bermudes, 1985).

2) The various eukaryotic intracellular motility systems (tubulin, actin-myosin based processes of pinocytosis, phagocytosis) are unknown in prokaryotes. Therefore, they may have originated by merger of the original spirochete with its bacterial host. In any case this is a eukaryotic seme.

3) The polyphyletic acquisition of mitochondria from respiring eubacteria seems to be established as a process that occurred in eukaryotic cell evolution (Gray, 1983).

4) The appearance of histone proteins as components of nucleosomes in the origin of chromatin occurred in the evolution of

several lineages of protists (Searcy and De-lange, 1980). Such nucleosome histones did not appear in the ancestors of dino-mastigotes.

5) The evolution of specialized cell organelles (e.g., extrusomes, hydrogeno-somes, methanogenosomes) occurred in eukaryotes, ancestors to modern protist (van Bruggen et al., 1985).

6) Studies of protist cell division reveal that mitotic microtubules, kinetochores and mitosis evolved in this group of organism (Margulis and Sagan, 1986).

7) Symbiotic plastid acquisition occurred polyphyletically in many groups of protists leading to the origins of various kinds of algae (Gray, 1983).

8) Colonial morphogenesis, chracteristic of several groups of protoctists (myxomy-cota, labyrinthulida) evolved in this group of organisms (Margulis and Sagan, 1986).

9) Cannibalism, diploidization and higher polyploidization can be understood as preadaptation for the origin of meiotic sex in protoctists (Margulis and Sagan, 1986).

10) The reduction of polyploidization, a preadaptation for the origin of meiosis, evolved in several protoctist lineages (Margulis and Sagan, 1986).

11) Alternation of haploid and diploid generations and the origin of embryogenesis are polyphyletic phenomena that made their appearance during the transition from protoctists to animals and plants (Margulis and Sagan, 1986).

This listing, as presented here, is of course immensely oversimplified. Yet by the last entry we have arrived at Mendelian genetic systems and the origin of animals. All of the seme acquisition listed here occurred in the pre-Phanerozoic, that is, prior to 580 million years ago, before the so-called «Cambrian explosion of fossils». When analyzed in this way it becomes clear that the Cambrian explosion of hard parts, the appearance in the fossil record of well-preserved fossils, simply made the previously subvisible complexity highly visible, analogous to the way abandoned automobiles are the manifestations in the fossil record of the level of development of the far less fossilizable hominoid neurocortex.

CONCLUSIONS AND PERSPECTIVES

The subject of the origin of life, or at least the origin of more manifest living beings, has fascinated mankind since the appearance of the earliest civilizations. However only in this century have we begun to understand enough biochemistry, genetics and paleobiology to develop a focused idea of the possible nature and antiquity of the first living entities. In this chapter we have seen that the salient characteristic of life is autopoiesy; we believe that the first autopoietic systems were prokaryotic cells, i.e., bacteria. The very earliest cells were probably of fermentative and phototrophic types, both obligately anaerobic. From the beginning of the history of life, such fermenters were most likely accompanied by primary producers having a photomixotrophic metabolism, i.e., organisms capable of utilizing both chemical compounds in their environment and sunlight as a source of energy. Even today we still have abundant living representatives of both types of obligate anaerobes. Certainly bacteria have evolved such that extant fermentative and photomixotrophic bacteria have many characteristics which were not requisite for the earliest functioning of their semes. During the first steps in the evolution of life many characteristics developed, both structural and functional; when the living world was only divided into animals and plants, these characteristics (e.g., oxygenic photosynthesis and respiration) were considered necessary conditions for the origin of any form of life.

During the Archean Aeon and at least half of the Proterozoic, life was exclusively prokaryotic. In the great kingdom of bacteria, organisms that are neither animals or plants, the essential genetic and metabolic mechanisms first appeared and diversified. On different occasions various sorts of prokaryotic cells associated to form communities which evolved into permanent symbioses. Such hereditary symbioses eventually developed into the different types of eukaryotic cells. These prokaryotic associations that became eukaryotes include microbial descendants that we recognize as the protoctists of today. The prokaryotic mechanisms of DNA duplication, recombination of genes and (in the case of symbiosis) of entire genetic systems, formed new autopoietic entities. These entities, bacterial and nucleated cells and their descendants, were subjected to immense selection pressures from the physical environment of the Earth's surface as well as from other autopoietic entities. With time the microorganisms (always as components of communities) expanded, recombined and reformed until they became the familiar macroscopic biota. We recognize today that nearly every major biochemical pathway present in animals and plants had already appeared in the cells comprising the Archean and Proterozoic microbes. No longer do we have to insist on a single solution to an immense number of problems hiding under the phrase «origins of life». Rather, we recognize that when we understand the origin of the first autopoietic entity, membrane-bound and capable of reproduction by division, we will in principle have come much closer to resolving the scientific problem of the origin of life.

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