Essays on Life Sciences, with Related Science Fiction Stories

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^{By} Pier Luigi Luisi

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FOREWORD

This book brings together articles published by the present author in the Wall Street International Magazine (WSI) between 2016 and 2018. Some of them are in their original form, others have been adapted slightly to ensure greater continuity between one article and the next. They are essays on life sciences, but perhaps rather than essays we should call them questions. Some are old questions such as: "What is life?", "What is death?", "What is cognition?", "What is consciousness?", "Why are macromolecules needed to enable life?", "Is our sense of beauty genetically determined?". Others respond to more recent dilemmas afflicting our world: "How can we solve our energy problems in an ecologically sustainable way?", "Is artificial intelligence at odds with our humanity?", and last but not least: "Are we alone in the universe?"

In the following pages I tackle thirty-odd questions of this kind, questions that have been asked and discussed many times before. But the reader will not always find me agreeing with mainstream contemporary thought—certainly not with respect to the misleading, indeed nonsensical, identification of life with DNA, or to current explanations of the origin of life on Earth, or to the hypothesis that there may be other civilizations within reach in our universe.

The volume consists of a series of essays arranged in three chapters, each of which concludes with a few science-fiction short-stories. Science fiction deserves a place here, because it is part of the life of our mind today. But these stories are not "mere" fiction: they are an extension of the essays themselves and raise serious questions which will confront humankind in the near future—questions that are not entirely new, and may, in many cases, never receive a definitive answer.

CHAPTER ONE

Foreword

We start from the origin of life on Earth. For science, this is an event determined and regulated by natural laws. Once divine intervention is removed from the picture, there is no other option. But this doesn't mean there are no more questions—quite the opposite. The mystery of the origin of life is still unsolved, and I doubt whether it will ever be solved, given the standard, present-day research approaches. There are good reasons why we should be sceptical. However, to be sceptical about our chances of finding a definitive answer does not mean we should abandon our research. One of humankind's most defining characteristics is its ability to ask questions small ones and big ones. And asking questions is more important than finding dubious answers. To inquire into the origin of life necessarily raises the issue of whether life may exist on other planets, a prospect that has long enthralled our collective imagination and will also be explored in this chapter.

Even if we do not know how life began, we do know that all life on Earth is cellular—without exception. Why should this be so? In order to come to grips with the phenomenology of cellular life, we draw upon the wellknown theory of autopoiesis proposed by Maturana and Varela, the socalled Santiago school. In my opinion, this theory is the only one capable of seriously tackling the question "What is life?". We then go on to ask "What is death?" and explore this question from a number of angles. At first glance this may appear a simpler question, but it turns out not to be the case.

After this, we change our perspective. Setting aside the outlook of western science, we turn to the oriental tradition, to Buddhism in particular. We join a colourful gathering of nuns in a Tibetan monastery in Bhutan and with them discuss what life is and what death is. We find that our scientific view of things is not necessarily perceived as absolute truth, especially when contemplated from a spiritual standpoint.

I. On the Origin of Life—With a Prebiotic Experiment

The origin of life is still an unsolved problem. We do not know how life originated on our planet, despite the work done in this field by excellent chemists and molecular biologists over the last 50 or 60 years.

Let's start from the beginning. We generally accept the view that everything started with the Big Bang over 13.8 Gya (billion years) ago, and that the solar system, and hence the Earth, appeared about 5 Gya ago. At that time the Earth was a fire ball, not an ideal condition for organic life! But as early as 3.8 Gya (some say 4) the Earth was rich in life in the shape of active unicellular organisms—microbes. The following figure illustrates the famous fossil bacterial form which is considered the earliest testimony of life on Earth (see for example Nutman et al., 2016).



Figure I.1. The first fossil cell (a), found in stromatolites in western Australia (c), and dated c. 3.8 billion years ago. The reconstructed model (b) looks very much like a modern cyanobacterium.

The question is: first there was no life on Earth, then Earth was teeming with life. How did life come about? This kind of question would not become a serious one till the middle of the nineteenth century. Before that, people were pious and good-natured and believed firmly that God had created the world and all living forms once and for all. It would have been blasphemy to challenge such a simple truth.

It took a British naturalist, Charles Darwin, in a book published in 1859, to turn blasphemy into science. Darwin rejected the idea that living forms were fixed, claiming instead that they have changed over time, and that they have evolved from a common ancestor. Piling blasphemy on blasphemy, he went so far as to argue that life might have originated from natural causes. Since then, philosophers and biologists have increasingly accepted the idea of a secular origin of life, based on natural laws.

In 1924 a brilliant Russian chemist, Alexander Ivanovic Oparin, (fig. I.2) much influenced by Darwin and by the Marxist dialectic materialistic philosophy of the Soviet Union, published a little book on the natural origin of life (Oparin, 1924). According to Oparin, life evolved from inanimate matter. Small molecules assembled into larger ones, and by a process of spontaneously increasing complexity and functionality, closed spherical structures (the first cells or protocells) were formed, and these were able to grow and multiply.



Figure 1.2. Alexander Ivanovic Oparin, who in 1924 wrote, in Russian, the first scientific book on the origin of life on Earth.

No divine intervention, no spirituality in this view of life on Earth! At least, not at the level of the first unicellular forms of life.

Oparin postulated that the prebiotic atmosphere was made up largely of four gaseous components: hydrogen, methane, water, and ammonia (H₂,

CH₄, H₂0, NH₃) and that through their combination, thanks to the energy provided by lightning, amino acids were formed. In the early nineteenfifties, an American Ph.D. student, Stanley Miller, fascinated by Oparin's idea, carried out a famous experiment. He filled a flask with Oparin's four gaseous components and fed electrical discharges through the flask to simulate the energy of primordial lightning (fig. I.3). What he witnessed was the formation of several α -amino acids and other biologically important substances. The experiment was published in 1953, (Miller 1953), the same year as the discovery of the double helix by Watson and Crick, a memorable year indeed for biochemistry.



Figure I.3: Stanley Miller and his famous experiment.

At this point a chemist might think: if life is only a matter of chemical interactions, and if the basic constituents of life can be synthesized under prebiotic conditions, then it should be possible to construct life in the laboratory... But—there is an important but—to have amino acids does not mean to have life. Amino acids make up all proteins of earthly life, but they are simple, non-living bricks. The same can be said for all small molecules, the so-called low-molecular-weight compounds, including mononucleotides, the monomeric constituents of nucleic acids. In fact, a chemist can have at hand all the low-molecular-weight compounds on this earth—amino acids, mono-nucleotides, lipids, sugars, metal ions, etc... and will still not be able to create life. To convince you of this, let's carry out an experiment together.

The Prebiotic Experiment

Let's take a 100-litre water tank, under strict sterile conditions, and add all the prebiotic chemical compounds we know of—amino acids, nucleotides, sugars, fatty acids, hydrocarbons—together with all the salts, metal ions and everything else our terrestrial crust may have possessed. We can add a thermostat, in order to change the temperature every week or so. There is a problem of concentration: our solutions may be too diluted. So, let's make two versions of the experiment, and in one of them, let's evaporate the water periodically so as to recreate the situation of a lagoon which has dried out, producing a very high local concentration of the compounds.

According to Oparin, lightning may have been the energy source that induced a reaction among the four basic components of the prebiotic atmosphere, thereby producing amino acids. Even if more recent literature has shown that these electrical discharges were not necessary, let's set things up so that each tank is equipped with electrodes that give electric discharges every few minutes, and so that a few microlitres of solution are automatically removed from each tank at intervals for analysis. Let's wait for some time to see whether any reaction products are formed. Shouldn't some protocell appear after a while? If this happened on prebiotic Earth, why not again?

Why not?

It can easily be predicted that nothing will happen. At the most, we might get the formation of vesicular structures from the fatty acids originally added, but no interesting macromolecular precursors, for instance orderly sequences of amino acids or polynucleotides. Perhaps the dry lagoon experiment will produce some random polymeric structures, but for them to achieve life, namely the mutual interaction between orderly sequences of amino acids and orderly sequences of polynucleotides, there is a very long way to go.

Experiments on the origin of life, conceptually similar to those mentioned above, have been conducted by chemists over the last fifty to seventy years in the best university laboratories in the world, using all the ingenuity of modern chemical knowledge. But to no avail. Self-reproducing protocells, or even protocells in a simple homeostatic regime, have never been obtained. If making life had been an easy process, scientists would already have produced it many times in the laboratory. It has never happened. Not yet at least. The question then is: why not? Why hasn't life formed spontaneously, considering that we have placed everything that Earth had to offer in prebiotic times in our reaction tanks? The most straightforward explanation is simply that the formation of life is not a spontaneous thermodynamic process. Which amounts to saying that the origin of life is not deterministic.

So, what is the alternative, if we remain within the framework of science? In terms of the classic controversy in life science, the alternative to determinism is contingency. Most of what has been written about contingency over the last fifty years has been in the field of the origin of life. Contingency (not to be confused with the related term "chance") is the notion that the evolution of a given process, instead of proceeding linearly from cause to effect—follows an erratic zig-zag pathway, due mostly to changing environmental conditions.

A crude representation of how contingency works is given in Figure I.4. (Luisi 2016). Obviously if the construction of a growing structure follows such a chaotic route, most of the processes will come to a dead end. Their final product will disappear and no record will remain in the history of evolution. However, some of these avenues of contingency may turn out to be useful, in the sense that they stabilize the structure itself, or give rise to a more resilient unity—possibly even something that is capable of self-reproduction.



Figure I. 4: The pathway of contingency in the idealized growth of, say, a peptide chain p (but valid for any other molecular-evolution growth) which encounters five different reaction partners, a, b, c, d, e. The choice of the reaction partner is not driven by chance (otherwise all possible products would be formed) but determined instead by the structural determinism of p, and by environmental conditions (like temperature, pH, concentration, pressure, dielectric constant...) which are independent of each other and of p. The same holds for the next step of the compound pb. The reaction partners a, b, c, d, e etc. are put there by chance, but the reaction's progress as such is not driven by chance.

We might argue that four billion years ago there perhaps existed unknown chemicals or unknown environmental conditions that we are unable to reproduce today. But this would be explaining one mystery by another mystery. We could also argue that for life to emerge it might take years or centuries for the reactions to take place. But this too is a weak argument, since even when reactions have a long induction period, we should still be able to observe the formation of some initial aggregates with our powerful analytical methods.

Accepting that there is no determinism in the origin of life enables us to account for a number of things. In fact, in a contingency pathway there is no way of predicting the causal succession of events, i.e. there is no predetermined logical connection between one step and the next. Even more importantly, it enables us to recognize that the origin of life is definitely not due to a single unitary process. We have no reason to assume anything of the kind, once we rule out the predetermined plan. It is much more likely that we are dealing with several independent pathways that crossed each other at a certain point, giving rise to a more complex web of contingent avenues. For example, we need to have the formation of orderly sequences of proteins, and the independent invention of orderly sequences of nucleic acids, followed by their mutual interaction, so that the sequence of one becomes causally dependent on the sequence of the other. But we don't know how any of these processes originated, or how they interlocked.

This is the crux of the problem: not only have researchers into the origin of life failed so far to reproduce in the laboratory the events which led to the origin of life, but they have also failed to come up with a scientific hypothesis as to how life may have originated.

Compare this situation to that of research into the origin of the universe. There we have the Big Bang theory, which doubtless contains elements of uncertainty and doubt—but the vast majority of scientists stand behind it, and it accounts for all or most of the cosmic data we know of. Whereas for the origin of life we have no such hypothesis. There are a number of speculations, the best-known being the "prebiotic RNA-world". However, it has been clearly demonstrated (e.g. in Luisi, 2016) that this does not make sense from the chemical standpoint.

Let me briefly restate the argument here. This hypothesis—in its most basic version—is based on the *a priori* existence of an RNA molecule which is capable of self-replication. But to get self-replication you need the formation of an RNA dimer, which implies a minimal starting dimer concentration of say, 10^{-10} M. This in turn would mean (the Avogadro number being what is it, i.e. 6.02×10^{23}) that in one litre of that prebiotic soup you would have to have roughly 10^{13} identical copies of that RNA. How could so many billions of identical copies ever come to exist? You need at least to have had the spontaneous formation of, say, a few picograms of that pure, functionally active RNA compound in the prebiotic tank. This is equivalent to invoking a miracle. There are other theories based on miracles that carry more weight...

The point, then, is this: our tank experiments just do not work, despite the innumerable ingenious variations of two generations of brilliant chemists. What is worse, we don't even have a persuasive theoretical hypothesis as to how life may have originated. We need to face these hard facts. Maybe tomorrow some brilliant bio-Einstein will discover the solution, but for the moment we simply don't have the slightest idea of how life arose from non-life. Of course, this doesn't mean that we should give up this research and resign ourselves to the existence of a mystery (a mystery is a problem that by definition has no solution). But it is crucial that we start out with a clear and honest theoretical position.

II. The Parable of the Green Man

We hear a great deal nowadays about sending space vehicles to far-away planets in the hopes of discovering some kind of life there. The famous Kepler-62 system shown in Figure 1.5 has six planets in the so-called habitable zone. All of them except 62C are larger than our Earth, but they are so far away that there is no plan to send probes in their direction. For the moment the question of whether we are alone in the Universe remains unanswered. NASA and other space agencies are multiplying their efforts at exploring Mars, the red planet, and such is their determination to find vestiges of former life there that they will no doubt end up by finding it. The question is: exactly what forms of life should these astronauts be looking for? They need to have a "definition of life" to start with, in order to distinguish life from non-life.

In the scientific literature we can actually find what is known as "the NASA definition of life". Originally, this was simply an operational perspective devised by the Exobiology Program within the National Aeronautics and Space Administration—a broad working definition originally proposed, it seems, by Horowitz and Miller way back in 1962. However, researchers into the origin of life began to use this as a definition of life as such, the kind of life we should be searching for. It states: *Life is a self-sustained chemical system capable of undergoing Darwinian evolution*.

This remains the most popular definition in the field even today probably because everybody understands it, just as everybody understands a simple tautology. If, on some distant planet, NASA astronauts happen to find a colony of bacteria that behave just like terrestrial ones, they will quickly radio home that, yes, they have found life! But as a definition, it does not say anything. It assumes *a priori* that life on other planets must be like life on this planet, as well as assuming that it is subject to a Darwinian type of evolution. It also tacitly assumes that the first forms of life we find, if any, are likely to be microbial. This is fine from a pragmatic viewpoint, since we incapable of thinking of anything different when we use the word "life".

But, of course, this cannot be considered a general solution to the problem. When the astronauts call earth to say that they have found cellular life, what if somebody asks them—*Yes, but what is life?*

The term "Darwinian" is particularly problematic. It refers to a population and is meaningless for a single item in isolation. The main point, I believe, is that we need a definition of life that is operative at the level of a single individual. Look at a dog barking or a dolphin swimming...and tell me why they are alive, here and now, without using the argument that they come from generations of Darwinian reproduction. If our NASA astronauts run into a single carnivorous monster, they may finish up eaten before they can find a corresponding population in which to study their evolutionary mechanisms. They well be victims of a mistaken definition of life....



Figure II:1 The Kepler planets, compared to our planetarian system.

From an epistemic standpoint, the Darwinian mechanism is actually a consequence of life, not its necessary condition. As already noted, the popularity of the NASA definition rests on an obvious tautology: life is Earth's cellular life, based on nucleic acids and their replication mechanisms. This aprioristic equating of DNA and life is, I believe, one of the main reasons, if not the only one, for our lack of progress in research into the origin of life.

Now let's tip all this upside down. Let's forget our NASA astronauts and consider instead the Green Man sent by the High Government of Alpha-Centauri to investigate Earth and specifically to clarify what life is on this planet. From his superiors he has received a long list of terrestrial things they don't know whether to count as alive or not. Our Green Man's job is to land on the Earth and clarify the situation. Let's see what happens.

He encounters an intelligent but scientifically naive farmer to whom he shows his list. The farmer quickly divides the Green Man's items into two columns, one of living things and one of non-living things. The Green Man is surprised at how quickly the farmer distinguishes between them and asks how he does it. He wants to know what property characterizing the living things in the left-hand column is missing from the items in the right-hand column.

Pointing at the *mule*, the farmer says "movement" and "growth". The Green Man nods, but with reservations, since neither trees nor coral in the living column move about or show any appreciable sign of growth over a reasonably long observation time. Conversely, a small piece of paper moves in the wind and the moon moves, as well as waxing and waning periodically.

THE GAME OF THE 1	TWO LISTS
LIST OF THE LIVING	LIST OF THE NON-LIVING
FLY	RADIO
TREE	AUTOMOBILE
MULE	ROBOT
BABY	CRYSTAL
MUSHROOM	MOON

QUESTION: what distinguishes the living from the non-living? IN OTHER WORDS: what quality or qualities are present in all items in the living list and are not, and cannot be, present in any of the items of the non-living list?

COMPUTER

Figure II.2.: The game of the two lists

CORAL

When the farmer suggests "reaction to stimuli" as an alternative criterion, the Green Man again nods somewhat doubtfully, since mushrooms and trees seem insensitive to a needle. On the other hand, a computer or a radio is inclined to stop working if you jab a stick into it. The farmer, beginning to get irritated, adds: "Living things take in food and can thus move around. Energy is transformed into action." But the Green Man points out that a car and a robot are able to move about by doing precisely that – converting energy into action.

"Reproduction!" cries the farmer. "All the items in this column are able to reproduce themselves!"

"As I understand things—says the Green Man after a short pause reproduction is a consequence of life, not its origin. In order to reproduce, don't you have to be alive first? Surely reproduction is a property of life, not its cause?"

Then, after a short silence, he adds rather sarcastically: "Also, reproduction is just fine for chickens and men, but not for a mule, which is unable to reproduce itself. Nor can babies, or old people. What's more, for mammals to reproduce takes at least two of a kind, whereas I want to know about a criterion for life at the level of a single specimen."

The farmer is beginning to lose his patience, but suddenly he has a brilliant idea. He looks at a tree and realizes that it loses its leaves and fruit in winter but generates them anew in spring—from inside itself. The same happens with his own beard and the hairs of animals: he cuts them and they grow back again. And all this happens thanks to an activity inside the body! The farmer also knows that when his pig is sick and can't eat, its limbs and organs get smaller. However, as soon as it starts to eat again, its limbs and organs grow bigger again. And this growth comes from inside its body. So, the farmer concludes – and tells the Green Man – that all the items in the "living" column have internal processes that continually destroy and rebuild the structure from inside itself. So, living organisms are characterized by an activity that regenerates their own components!

This time the Green Man nods positively. The farmer has finally defined the quality that distinguishes the living from the non-living! The robot, computer, radio, moon, and so on, are not able to regenerate themselves from the inside. If a part in a radio breaks, the radio itself is unable to repair it. Whereas all items in the left-hand column do have this quality: they utilize external energy to maintain their own structure and are capable of regenerating their structure from within themselves. This seems to be the property of life that he is looking for.

The Green Man now draws a figure on the ground (Figure II.3). It shows an entity that is open to the medium surrounding it. S stands for a component of the living system which is being transformed into a product, P. The system is able to regenerate S by transforming the entering food A back into S.

The Green Man is rather pleased with this. So, he and the farmer put together the following "operational" definition of life:

A system can be said to be living if it is able to transform external matter/energy into an internal process of self-maintenance and production of its own components.



Figure II.3. The simple drawing of the Green Man, which represents his understanding of life on Earth.

The farmer nods, though I doubt whether he fully understands. Yet together they have arrived at a "definition" of life by using macroscopic, common-sense observations. Such a simple definition might easily have been worked out by laymen a couple of centuries ago. You don't need molecular or cellular biology for that. The definition was derived from a single specimen but has general validity, and thus holds for both coded life and non-coded life.

The farmer was ignorant of biology, otherwise he would have answered right away that all living things are made up of cells—that this is their main distinguishing feature. If he had done so, however, the Green Man would have asked: "What is a cell and why do you call a cell alive?".

The life of a cell is, in fact, the starting point for the development of the idea of *autopoiesis* (from the Greek *auto*, or self, and *poiesis*, making, producing) developed by Maturana and Varela from the mid nineteen-seventies on (Maturana and Varela, 1973, 1980, 1998; see my essay on autopoiesis in Chapter 2).

The theory of *autopoiesis* tackles the question "what is life?" and attempts to isolate, above and beyond the diversity of all living organisms,

a common denominator enabling us to distinguish between the living and the non-living. Autopoiesis is not directly concerned with the origin of life. Rather, it is an analysis of the living in its here and now, as the authors put it. As we will see, once the question "what is life?" is clarified by the theory of autopoiesis, this will enable us to pose the question of the origin of life more correctly. We will come back to this concept in the following essay.

III. Reversing the Bottom-up Approach to the Origin of Life

Introduction

We are surrounded by systems of astonishing complexity-from the immensity of the cosmos with its stars, galaxies, and black holes, to the intricacy of life in its various forms. We generally assume that this complexity has its roots in simpler structures which, in the course of time, accumulated material until they achieved their present form. As regards the origin of life on Earth, we have already discussed Oparin's thesis that life developed from non-life, the original simple components of the prebiotic biosphere combining to form the first amino acids and nitrogen-containing compounds. Subsequently, these first molecules, combining with each other according to simple natural forces, gave birth to more and more complex molecular architectures leading up to the formation of self-replicating cells-the basic condition for life as we know it now. Hence the usual approach to the origin of life and of cells is essentially a bottom-up conceptual and operational procedure, according to which present complexity has its origin in simpler, primitive forms. Materials are added, piece by piece, thereby bringing about a complex whole.

The Bottom-up Approach to the Minimal Cell

Since Oparin, many teams of distinguished scientists have engaged in projects aimed at understanding the origin of life on Earth, including trying to reproduce molecular evolution's pathway to life in the laboratory. Implicit in all these projects is a bottom-up approach to the natural transition to life. This kind of assumption also underpins most synthetic biology, especially the construction of the so-called "minimal cell". This is exemplified in Fig. III.1: essentially it involves using vesicles (generally liposomes, i.e. vesicles formed by phospholipids) into which enzymes and nucleic acids can be incorporated. But how many? The basic idea was to entrap the minimum sufficient number of genes and enzymes so as to produce a minimal cell.

This work was begun in the nineteen-nineties, mostly at the ETH in Zurich, by Thomas Oberholzer and others in my research group (Oberholzer et al., 1995-2002). Since then, several groups around the world have worked on the biochemistry of enzymes and nucleic acids entrapped in vesicles (for a detailed review of the data see Luisi 2016). However, in all these experiments, it has proved impossible to entrap more than a couple of

different enzymes in one compartment, and all these systems, although representing an important new concept in synthetic biology, are really too simple to be considered close to biological cells.

A qualitative leap in this field was brought about by the discovery and commercialization of the so-called *Pure System* by the Ueda group (Shimizu et al., 2001, 2005). It consists of a minimal transcription-translation system, containing 37 enzymes and a total of about 90 macromolecules, together with a series of small molecules, including ATP (adenosine triphosphate, which provides energy for the reactions). It was possible to entrap the entire system in vesicles.



Figure III.1: The operational and conceptual approach to the minimal cell, whereby the cell's final complexity should be achieved step by step through the addition of functional macromolecules. The minimal quantity of these necessary to ensure viability is what defines the minimal cell.

This is important, because it shows that even large systems can easily be incorporated. Thanks to Ueda's *Pure System*, several groups around the world were then able to express the green fluorescent protein (GFP) inside vesicles under various conditions. GFP is used for detection purposes, since its formation gives rise to a green colour. Nevertheless, it is fair to say that we are still a long way from a living, self-maintaining, biological cell. Put simply, the *Pure System* cannot reproduce itself. As a result, most of these studies relate to one-batch reactions.

From this point of view, the prospect of a self-sustaining cell, or the selfreproduction of the GFP-forming vesicle systems, doesn't appear realistic as yet. And as regards the complexity of a real cell, another quite different angle has to be taken into account, namely the research on the minimal genome. Such research shows that the minimal genome required to sustain a modern type of biological cell cannot have less than 200 genes, and probably requires around 250 genes. In addition to these theoretical studies, experimental work based on the "knock-out" technique recently developed by the Craig Venter group shows that you can simplify the genome of *Mycoplasma genitalium* but that you cannot go below 200 genes (Smith et al., 2003). Now, if we compare this figure of hundreds of genes with the experimental results and possibilities of the bottom-up approach to the minimal cell (Figure III.1), the empirical conclusion is that the bottom-up approach will never be able to reach a threshold as high as 200-250 genes.

As I've argued, the idea that the origin of complexity in nature and in life itself is due to a stepwise increase of molecular architecture is something almost innate in us—perhaps an archetypal, Jungian form of thought. But suppose this isn't the right approach? Then: what are we left with?

The Alternative to the Bottom-up Approach

Suppose we start from the top, i.e. with a large population of compartments that have been randomly filled with biopolymers. We then would have to assume, of course, that mixtures of nucleic acids and proteins already existed from the very start- an assumption usually unheard of. In fact, we have no indication that this was the case, but let's assume that they must have existed someplace in quite large amounts.

What do we need then to arrive at the conditions for making a cell? We need surfactants, so as to make membranes, and we also need an efficient method for entrapping biopolymers in the vesicles. Lastly, we need a high enough local concentration—as in our modern cells.

It so happens that it has been shown experimentally that these two apparently very stringent conditions—high entrapment efficiency and high local concentration—can actually be produced in the laboratory.

In fact, let's recall here the phenomenon of spontaneous macromolecular overcrowding in vesicles, which was first described by my team in 2010 at the University of Rome 3 (Luisi et al. 2010) and in later papers of ours. What it amounts to is this: when in a diluted solution of macromolecules, vesicles are produced *in situ*, the solute distribution in the vesicles does not follow the expected classic (Poisson-type) distribution.



Figure III.2. Some TEM micrographs showing macromolecular overcrowding, assembled mostly by Pasquale Stano and Teresa de Souza (2011, 2012). Data refer to ferritin as a guest protein molecule (which can be evidenced in cryo-TEM experiments thanks to its high density); the micrographs also refer to ribosomes, and RNA-polypeptide complexes as guest molecules.

Instead, we get a kind of all-or-nothing situation, with a lot of empty vesicles (i.e., that contain no biopolymers) and a few over-filled vesicles, in which most of the solute is packed, with concentrations up to 60 times higher than in the bulk solution. Figure III.2. illustrates some of the archive data. Consider that even when the frequency of overcrowded vesicles is as low as 0.5%, in one litre of that solution—given the Avogadro number—we find several billions of them (typically, given the initial concentration of surfactant, a total number of vesicles close to 10⁹-10¹¹, i.e. from one to one-hundred billion).

We come now to our second main assumption: that given the enormous number of overcrowded vesicles, there will be a least one "good vesicle" which has the right ingredients and the right concentration to start life, or, more precisely, for it to take the first dynamic steps towards life. There's no room here to describe possible routes of one such a mechanism. But the point I want to stress is that this mechanism provides the basis for a possible reversal of the bottom-up approach. What I am proposing is a systemic view. Multiplicity is key: i.e. the presence of a large number of vesicles, so large that we can reasonably assume that at least one of them will be viable.

This is a far cry from the bottom-up approach which assumes that, starting from one individual cell-like structure, we can increase its content until it finally achieves the complexity of the genome. In the systemic approach, on the other hand, complexity is present from the outset. The fact that the entire *Pure System* can easily be incorporated into vesicles demonstrates the reliability of this kind of proposal.

This is *not* a top-down approach: it is not a matter of scaling downward from complexity, but of adjusting initial complexity by means of selection and self-organized re-equilibration so as to arrive at a quasi-homogeneous population—potentially a colony—of neighboring and eventually viable vesicles.

So far, this is still a hypothesis, but one that provides a possible basis for experimental confirmation. One kind of experiment should aim to identify the conditions in which overcrowded vesicles interact and fuse with each other in order to reach an equilibrium, a homogeneous state—possibly in the form of a colony.

Certainly, the assumption of an original reservoir of DNA/proteins in prebiotic times is far from getting favors. We prefer to think that the 6 billion odds tons of DNA present in our planet have originated from the biogenesis of living organisms during evolution-all of them. Most probably, is like that. Or perhaps, not.

The main purpose of this essay, then, is to encourage younger researchers to look beyond the simplistic, bottom-up approach to complexity. There is another reason why this alternative approach may appeal to some biochemists. In fact, the idea that life may arise from the fortuitous existence of one structure among a billion of closely related ones, bears an intriguing resemblance to the so-called anthropic principle, according to which the emergence of the Earth as the home of life depends on an extremely fortuitous combination of the numerical values of cosmological constants.

One last point. The scenario outlined here also seems to be in tune with some modern views of the physics of self-organized criticality, according to which, as Peer Bak and collaborators (1987,1988) explain:

... dynamic systems with extended spatial degrees of freedom naturally evolve into self-organized critical structures of states which are barely stable... The combination of dynamic minimal stability and spatial scaling leads to a power law for temporal fluctuations...

Here—above and beyond the obvious differences between our biological system and Peer Bak's criticality conditions—we see that dynamic systems tend towards a final state, which is not a state of thermodynamic stability, but a process which can be formally described in terms of what is known as "power law" (a rather difficult concept. Perhaps the Internet can help you out with that...).