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The System View of the Origin of Cellular Life

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Abstract

The commonly accepted view in the system biology of the minimal cell is a bottom-up approach which assumes that one can reach the properties of modern cells by stepwise increasing the complexity of biopolymers-containing vesicles. It is argued here that the great gap between the paucity of the results achievable in such a way, *vis a vis* the complexity of modern minimal cells, opens the question of the validity of this bottom up, stepwise approach as a matter of principle. We present here arguments in favor of an alternative view point, a systemic approach starting from the top, namely with the whole system of a very large population of mutually interacting vesicles initially randomly overfilled with DNA, RNA, proteins. This view is based on published literature data reporting the spontaneous overcrowding of vesicles formed *in situ* in a diluted solution of biopolymers, once that there is the simultaneous occurrence of vesicle-forming surfactants in the same macromolecular solution [28-32].

The assumption is then made, that if such an enormous number (10^9-10^{11}) of overfilled vesicles would be formed, then there would be a finite probability that one of them, or even a few, could have the right combination and concentration to start life – or at least the first dynamic steps towards a selection process, which, through mutual interaction, fusion and eventually vesicles proliferation, could arrive at a homeostatic equilibrium, conductive to the first forms of cellular life – possibly already at a level of a quasi-colony.

Introduction

The general approach to the origin of life and to the origin of cells is basically a bottom-up conceptual and operational procedure, according to which the present complexity has its origin from simpler, primitive forms. Thus, we reason that life could not have started right away with cells containing thousands of genes, and the way of synthetic biology to the minimal cell, supposedly mimicking molecular evolution, foresees the incorporation of one or very few biopolymers at a time inside a vesicle, increasing the number of components until

we reach the complexity and functions of the living cell.

However, this bottom-up approach to complexity – despite a number of per se interesting papers – did not achieve as yet a great success. The basic questions of the emergence of life from non-life, and the emergence of the first functional cells, are still unsolved problems, with no clear sight of a way out. But precisely this paucity may open the question, whether the stepwise bottom-up reasoning is necessarily the correct one. The present paper, based also on experimental observations which have been made in the last few years, wishes to present an alternative, systemic way of thinking. It is perhaps necessary to stress again, that there is nothing new in the data, it is however a radical new way to interpret them and with them, the whole concept of bottom up approach to the origin of life.

The synthetic biology of the minimal cell

Let us go back to the challenging approach in synthetic biology about the incorporation of biopolymers, and in particularly enzymes, in vesicles or liposomes (i.e. vesicles formed by phospholipids) in order to make models of a compartmentalized biochemistry. The interest was increased by the demonstration that vesicles, just like aqueous micelles [1], could undergo self-reproduction [2-3]. All this became even more interesting when it was shown that also active ribosomes could be entrapped in liposomes [4], and in fact this opened the way to the synthetic biology of the minimal cell, broadly defined as the cell-like system containing the minimal number of macromolecular components [5-7]. Already Harold Morowitz, in his 1992 *Beginning of Cellular Life* [8], had expressly formulated the hypothesis that the first step towards the origin of life might have been the formation of vesicles as first protocells. Following all that, several groups all around the world became active with the biochemistry of enzymes and nucleic acids entrapped in vesicles – and a number of initial, important papers were published: for example, the synthesis of Poly(A) by enzyme entrapped in vesicles, also with the possibility of simultaneous vesicle self-reproduction[3], or the replication of RNA by the enzyme Qβ-replicase [9], even in self-reproducing vesicles[10]. It was even attempted to enzymatically synthetize phosphatidylcholine (PC) inside PC liposomes [11] in an attempt to self-reproduce vesicles from the inside. However, in all these experiments, one could not 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 very close to biological cells.

Even the above-mentioned systems in which nucleic acid (RNA) was produced inside self-reproducing vesicles, or the older system in which lecithin was allegedly produced inside lecithin liposomes, are still one-batch system, and do not correspond to a homeostatic system of life. There was a qualitative jump in this field with the discovery and commercialization of the so-called Pure System by the group of Ueda [12] in 2001. This is a minimal transcription-translation system, containing only 37 enzymes and a total of ca. 90 macromolecules, with a series of small molecules including ATP. It was possible to entrap the entire system in vesicles.

Several groups around the world were then able to express the green fluorescence protein GFP (GFP for obvious detection reasons) under various conditions

[13-18]. Those include the extreme cases of very small vesicles [18] (only 200 nm in diameter) – even with the observation of enhanced protein synthesis inside liposomes – and the case of giant vesicles [15-16]. Still, it is fair to say that we are still far away from a living self-maintaining biological cell.

In fact, the Pure System cannot reproduce itself, and therefore most of these studies are relative to one-batch reactions, and the perspective of a self-sustaining cell, or of a self-reproduction of the GFP-forming vesicle systems, does not appear as yet as a realistic scenario. Not even with an ensemble of 37 enzymes. And there is another, quite different angle to consider: the studies on the minimal genome. First of all, the smallest possible genome found in unicellular organism in nature consists at least of 500-600 genes – as in the famous example of *Mycoplasma* genitalium (580 kb), or Buchnera *spp.* (450 kb). And furthermore, as discussed by a series of theoretical biologists over the years, like Mushegian and Koonin [19-21], Shimkets [22], Gils [23-25], the minimal genome to sustain a modern type of biological cell cannot have less than 200 genes, and more likely should be around 250-260 genes. In addition to these theoretical studies, there is the experimental work based on the technique of knock-out, more notably, in recent times, by the group of Craig Venter. This is a valuable work which indicates that the minimal number of genes in the case of *Mycoplasma genitalium* can indeed be considerably reduced, but certainly not below 200 genes [26-27].

Now, if we compare the figure of hundred genes with the experimental results and possibilities of the bottom-up approach to the minimal cell, a basic question comes to mind – a question which actually invests all our thinking habits within the molecular evolution of life: whether our conceptual and operational bottom-up approach is meaningful. The idea that the origin of complexity in nature and of life itself is due to a stepwise increase of molecular architecture is something almost inborn in us, perhaps an archetypical Jungian form of thought. But we should wonder whether this way of thinking is correct, or, at least, whether some alternative pathway is possible. Of course, one could object the following: the fact that we have not been able to perform the stepwise complexity approach in the lab, does not rule out the possibility that this process has taken place in nature. But on the other hand, why do we have to stick to this reductionist, simplistic way of seeing the origin of complexity? If we for a moment forget the bottom-up approach, what is left?

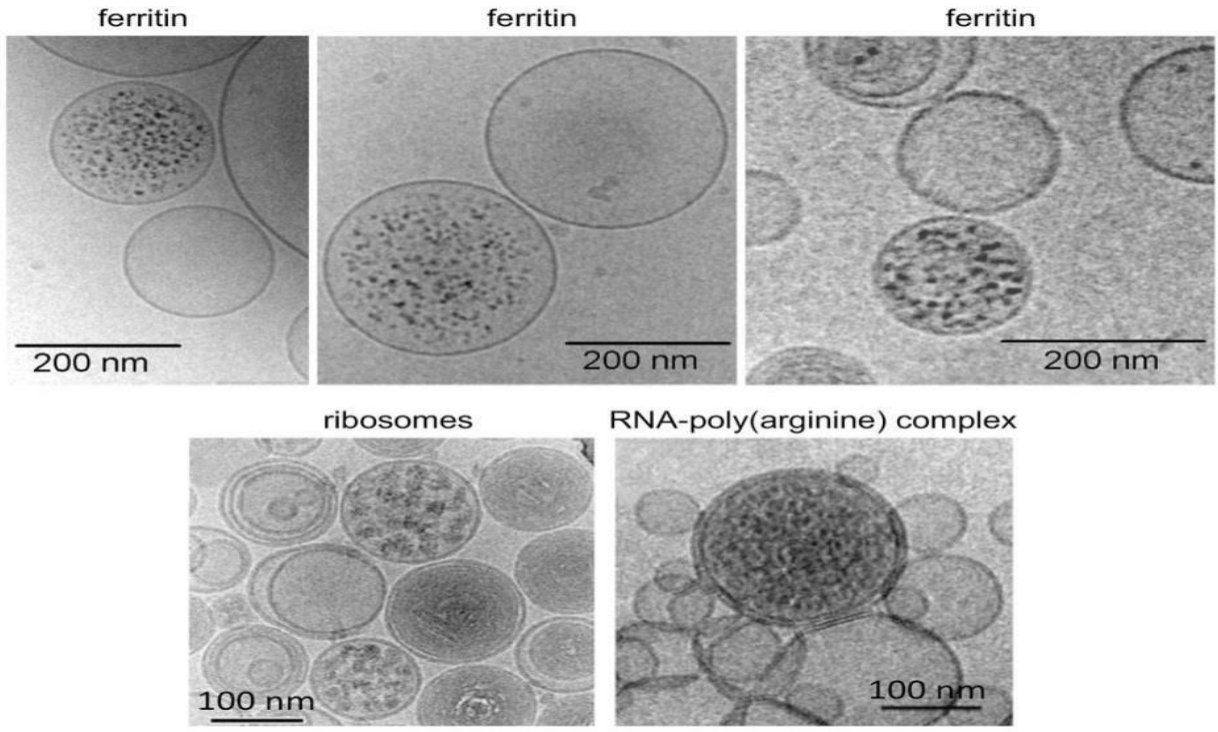
Spontaneous macromolecular overcrowding

What may come to mind is to start from the top, namely from an extant population of compartments randomly filled with a lot of biopolymers. We have to assume of course that mixtures of nucleic acids and proteins are already existing; and still we do not know how they were formed in prebiotic time, but we know that they were certainly there in that time, and most likely not in tiny amounts; and we have also to assume that the local concentration, at least in some of these compartments, was high enough to permit reactivity. The prerequisite of the entrapment in vesicles with an appropriate high local concentration may appear particularly disorienting. However, to this aim, one should call to mind the reports on the spontaneous macromolecular overcrowding in vesicles, described first [28] in 2010 and then in a few following papers [29-32].

This is the following: when in a diluted solution of macromolecules, vesicles

are produced *in situ*, the solute distribution in the vesicles does not follow the expected Poisson type of distribution. Instead, there is a kind of all-or-nothing situation, with a lot of empty vesicles (i.e., not containing biopolymers) and a few over-filled vesicles, in which most of the solute is concentrated [28-32]. The final local concentration of macromolecules inside such "overcrowded" vesicles may be at least one order of magnitude higher than in the original bulk solution [29-32] up to 60 times higher [29] in some case. This has been first documented with ferritin, a large high-density protein which has the advantage to be "seen" as a single molecule at a time in crio-TEM experiments; and later for other macromolecular systems, including ribosomes, nucleic acids, and even simple synthetic polymers [29-32]. (Figure 1) illustrates some of these archive data, collected mostly by Pasquale Stano and Teresa de Souza.

Figure (1): Some TEM micrographs showing the macromolecular overcrowding, collected mostly by Pasquale Stano and Teresa de Souza (see cited papers [29-32])

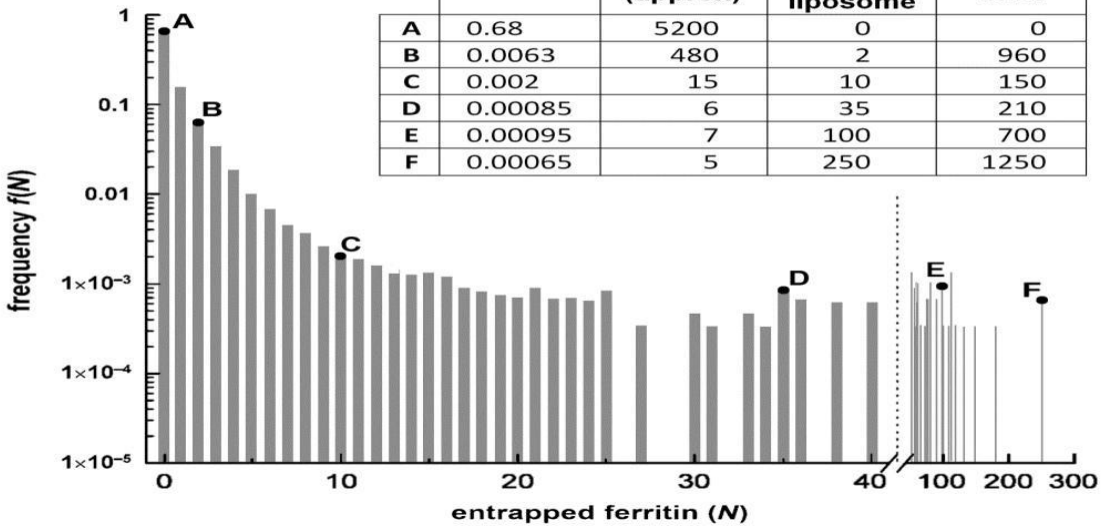


Let us consider now (Figure 2), which is a histogram rendering of the single points presented in the cited papers [29-31]; the enclosed table reports, as example, a few numeric data. Both histograms and the table in this figure shows the average data coming out from several experiments (we have hundreds of them in our archive), and it is evident that there are many "empty" vesicles, and a few very concentrated ones. In particular: 95% of the liposomes contain only 0-2 molecules of ferritin, with a total of ca. 17% of the solute; there is an intermediate class; but then 0.5% of the liposomes contain up to 100-250 molecules of ferritin each, for a total of ca. 80% of the material. Also, consider

that even when the frequency of overcrowded vesicles is as small as 0.5%, in one litre of that solution – given the Avogadro number – we have several billions of them (typically, given the initial concentration of surfactant, 10_9-10_{11}). This figure will become relevant shortly. The many data together, collected with four different concentrations of ferritin, far from displaying a Poisson-type of distribution, follow a kind of power law [30-32]. The mechanism of this deviation from a Poisson distribution has been the subject of preliminary theoretical considerations [33], but it is not yet completely clear, and is probably due to a concomitance of kinetic and thermodynamic factors.

Figure (2): Observed frequencies, ***f(N)***, of entrapped molecules of ferritin in liposomes. Histograms show average data collected in several experiments involving around 7,700 liposomes and starting from four different initial concentrations of ferritin (4, 8, 16 and 32 μM). The table shows (as example) some typical experimental results. Most liposomes (ca. 5,200, about 68% of total liposomes) do not contain any entrapped ferritin molecule (histogram **A)**. Many liposomes contain a few entrapped ferritin molecules (histogram **B** gives the numeric values for liposomes containing 2 molecules of ferritin). Point **C** shows an intermediate condition (a few liposomes containing a few entrapped molecules). By converse, there is a small frequency of liposomes (see examples **D**, **E**, and **F**) containing many entrapped ferritin molecules. (The graphic rendering of the single points was presented in the cited papers [29-31]). The total number of these liposomes is however of the order of 10_9-10_{11} per litre, given the typical initial concentration of vesicles (e.g., POPC at 1-2 mM) and the Avogadro number

	Frequency *f(N)*	Number of liposomes (approx)	Entrapped molecules of ferritin	
			in each liposome	total
A	0.68	5200	0	0
B	0.0063	480	2	960
C	0.002	15	10	150
D	0.00085	6	35	210
E	0.00095	7	100	700
F	0.00065	5	250	1250



The fact that a high local concentration permits biological reactions which are not possible in a dilute environment is well understandable, and it has been beautifully demonstrated [29] by diluting a commercial *in vitro*-protein synthesis preparation, so as to obtain no viability; and then adding vesicles-forming surfactant, thus recovering viability. The net result is rather clear, and important, given a diluted solution of biopolymers, the production of vesicles *in situ* is going to

produce cell-like compartments having a high concentration of reagents – so as to possibly permit interactions and reactivity which are not possible in a diluted solution. There will be a very high number of overcrowded vesicles, and they will differ from each other stochastically in terms of distribution and relative concentration of the reagents.

The systemic view

Now, let us elaborate on these figures in terms of a possible origin of cellular life. Possible links to an origin of metabolism have been already mentioned [30-31], but without considering the limitations of the bottom-up approach and the corresponding epistemic implications. So, let us start with an aqueous solution – the famous prebiotic lagoon – containing all the basic macromolecular components, with the further assumption of the simultaneous presence of a vesicle-forming surfactant (hot springs?).

Given this starting scenario, we would arrive at the situation described above in (Figure 2), with a solution containing billions of overcrowded vesicles with all the basic macromolecular components, and differing the one from the other in the stochastic distribution of material inside them. And now the main assumption: that given this enormous number of overcrowded vesicles, there is a least one "good vesicle" – perhaps a small family – which has the right ingredients and the right concentration to start life. Or better, and more simply, to start the first dynamic steps towards life. What this could be? Possibly self-reproduction at the expenses of macromolecular monomers in the environment, and thanks to the catalytic help of some protein(s) – as schematically illustrated in the following equation: Equation 1:

$$((N_gP_g)) + N_j + P_k = 2\,((N_gP_g)) + R \quad \text{(Equation 1)}$$

where the double parentheses indicate the vesicle, N and P nucleic acids and proteins, respectively, the index g referring to the "good vesicle" which can self-reproduce, and R the amount of material needed for stoichiometry. Notice that this is the beginning of a non-linear chain reaction, as two good vesicles will make four, and then eight, and so forth. Furthermore, since in a typical overcrowding experiment most of the macromolecular material is sequestered in the overfilled vesicles, the monomeric macromolecules Nj and Pk could come from the other sterile vesicles, via a mechanism of fusion with the good one. In other words, the good vesicles can destroy and transform the sterile ones – the great majority – into good vesicles, again in a non-linear chain reaction process. These first steps might consist then in mutual fusion, re-equilibration and proliferation processes which bring towards a more ordered equilibrated system – a system with quasi homogeneous vesicles in terms of content and mutual reactivity. The final system can also reach, in principle, a situation of homeostasis, given enough material in the environment.

This is then a systemic view: what is essential is the multiplicity, the great number of components that permits the reasonable assumption that at least one of them can be the right one – and following that, the mutual interaction of the components (the vesicles of the system) with each other, so as to arrive to a more homogeneous situation. Quite a difference with respect to the bottom-up approach, in which, starting from one individual cell-like structure, one attempts has to increase its molecular complexity so as to arrive at the complexity of the genome. The system view is a hypothetical pathway, in the sense that the experimental evidence for the fusion and re-equilibration of vesicles is still

missing. However, the described hypothetical scenario also gives a clear indication on how to possibly proceed experimentally.

In fact, although phospholipid vesicles and also fatty acid vesicles do not easily fuse with each other, there are indications in literature on how the fusion can be aided, for example with the help of surface charges [34-35]. And Yomo's group reported the fusion of giant vesicles containing DNA, the other a kit for protein expression [36-37]. A very relevant to this kind of scenario are also the data of fusion and proliferation of cells which are wall-deficient [38-40], a situation which is clearly similar to the case of vesicles. There are also clear literature indications on how an assembly of vesicles can be stabilized [32], and this would be important not only to facilitate interaction and equilibration, but also to form a kind of primitive colony. In fact, this is another advantage of this systemic view. Bacteria, archaea, and some other unicellular organisms do not live all as single entities, but also in colonies. Then, why do not start the quest of the origin of cellular life from a primitive form of colony?

Equation 1 is only one possible way to start the process, but actually one could advance other hypothetical pathways on how the initial mechanistic steps may be rolling out, but we do not want to go into this – the main point here is to indicate that there is a reasonable view which is alternative to the stepwise bottom-up way of thinking, once we make the fundamental assumption, that, given the extreme large number of overfilled vesicles, there is at least one, or a small family, which has peculiar reactivity.

In my recent book [41] I mentioned the analogy with the anthropic principle (AP) of the origin of our universe [42-44]. This may sound far-fetched and in fact it should be taken as a light metaphor. However, the analogy is intriguing: as is well known, the AP argument goes as follows: that not one, but very many-billions universes have been originally created, each one with its own cosmic constants and cosmic laws; and that in one of them happened to exist the right values to start life. In our case, there would be billions of overcrowded vesicles, with at least one of them which would have the right combination of reagents to start the initial steps conductive to life. Aside from the speculative character of such argument, there is an important general point: that nature, and probably also evolution, does not like to produce one single object at a time, but rather a great multiplicity (and this must be so also for the production of nucleic acids and proteins). And then work with selection. The scenario exposed here appears to be also in keeping with some of the modern views of the physics of the self-organized criticality, according to which, as Peer Bak and collaborators [45] state:… dynamical 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 – beyond the obvious differences between our biological system and Peer Bak's criticality conditions – we find the tendency of dynamic systems towards a final state, which is not a state of thermodynamic stability – with a formal description of the process in terms of power law.

Conclusions

Thus, the idea of the system origin of cellular life permits interesting conceptual relations and analogies with general natural laws – but also, more pragmatically; it also

brings a new way to look at the origin of cellular life. The main advice is this: as long as the origin of life is tantamount to the origin of cellular life, just forget the bottom up approach to the origin of cells. The criticism to the bottom-up approach should not be taken as a denial of the relevance of the synthetic biology work of the entrapment of biopolymers inside liposomes – actually this work has been important to open a new page in the biochemistry of compartmentalized enzymes and nucleic acid, showing emergent properties and possible biotechnological applications. The point is, that this procedure can never arrive at a real living cell, and that most probably this was not the way how nature fabricated complex cells.

What has been proposed is a systemic view, in which complexity is there from the beginning. The fact that the entire Pure System can be easily incorporated into vesicles demonstrates the reliability of the proposition. This is not a top-down approach, as the point is not to escalate down from complexity, but to adjust that starting complexity by way of selection and self-organized re-equilibration, so as to arrive at a quasi-homogeneous population – potentially a colony – of neighbouring and eventually viable vesicles. It is until now a hypothesis, but one that provides indications about possible confirming experiments. As already mentioned, one of them is to find the conditions by which the overcrowded vesicles interact and fuse with each other, so as to arrive at an equilibrium homogeneous state – possibly in the form of a colony; the other, on a quite different direction, would be on the side of geology, to look for hot springs which may give rise to vesicles-producing surfactants. More generally, the main message of this paper is an invitation to look beyond the simplistic bottom-up approach to complexity, and to consider the alternative.

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