Role of lipid membrane–nucleic acid interactions, DNA–membrane contacts and metal (II) cations in origination of initial cells and in evolution of prokaryotes to eukaryotes

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Abstract

The problems of the origin of primary cells and eukaryotic cells are discussed in terms of possible role of interactions between nucleic acids with lipid membrane according to corresponding original hypothesis. We propose that there are two main hypotheses of the origin of primary cells: (a) RNA appeared before proteins and DNA [Nature 213 (1967) 119]; (b) it is needed for the appearance of a primary cell, the volume closed by the lipid membrane. There was no information about the ways on how RNA appeared inside that volume for saving the reaction products around. Our hypothesis suggests that one of the starting points in the origination of primary cells was the interaction of nucleic acid and lipid membrane bubbles in the presence of metal (II) ions (which existed in high concentrations in prebiotic conditions), and this resulted in the enclosing of the pro-RNAs inside the lipid membrane. This hypothesis is formulated by us on the basis of experimental biochemical and biophysical studies of the DNA/RNA–phospholipid vesicles interactions in the presence of metal ions (II) fulfilled in the Institute of Biomedical Chemistry, RAMS, Moscow and Institute of Biophysics, RAS, Pushchino. Our belief is that DNA–membrane contacts (DNA–MCs) played an important role in the prokaryotes-to-eukaryotes transition. The model of the confluence of four prokaryotic cells may explain the prokaryotes-to-eukaryotes transition by the way of eukaryotic nuclear pore formation from prokaryotic Bayer contacts. The main requirement for the following fusion of prokaryotic cells must be their mutual orientation. After possible association, the division of the formed cell is begun. The great advantage of the model of four prokaryotic cells is the profit in the metabolism and the possibility of the intensive growth of intercellular membrane structures.

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1. Introduction

1.1. Hypothesis on the origin of the first cells

The interest to the research of the interactions of nucleic acids (DNA and RNA) with lipids and phospholipid membranes has greatly increased recently. This type of fundamental interactions of the main classes of biomacromolecules along with (phospho)lipid–nucleic acid interactions [1–3] and nucleic acid–lipid membrane recognition [4] becomes important during the consideration of the problems, connected with the nuclear matrix, regulation of the gene expression and gene transfer through the biomembranes [5–7]. It is established now that the DNA and RNA interact with membrane structures both in prokaryotes and in eukaryotes [8,9]. In both types of cells, DNA–membrane contacts (DNA–MCs) serve as the place of initiation of the transcription and replication, causing the higher expression of genome sites, contacting with the nuclear membrane. This increased interest is also connected with the recent use of the cationic lipid–DNA complexes (genosomes or lipoplexes)—for gene transfer and delivery for the purposes of gene therapy [10–12]. On the other side, this phenomenon is caused by entering molecular and cell biology (decoding human genome sequence [13]) to the “post-genome era”.

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with the increased interest to study the lipids' role in gene/ genome expression and regulation. The fact is that the hopes of the genome medicine to explain human diseases by genetic mistakes have reached a deadlock, because with about 3000 hereditary diseases, the number of all diseases amounts to some dozens of thousands. Thus, it is becoming obvious that the majority of the pathological states originated from the disturbances in gene regulation, but not by the defects in the gene structure. Up to now, it has been well established that chromatin-bound lipids (weakly or strongly DNA-bound lipids: cardiolipin, diglycerides, cholesterol and its ethers) play a great structural and functional role, and they are involved indeed in the regulation of gene expression [14]. Taking into account these findings, we proposed that lipid-like molecules and their interactions with the pre-nucleic acids could play a key role both in evolution of prokaryotes to eukaryotes [15] and in origin of initial cells [16]. Currently, the problems and questions on the origin of life and functioning initial cells are of great interest not only for the chemists and biologists, but also for the wide scientific community [17–32].

Current opinions in the origin of initial cells could be summarized using the following ideas:

(a) The initial cells originated in the World Ocean at a depth of a few meters—they had to be protected from the solar radiation due to the absence of the ozone layer in the atmosphere [33];

(b) According to Crick's hypothesis [17], mediators of genetic information in initial cells appeared to be pro-RNA molecules, capable of replication and protein synthesis (those molecules appeared before proteins) [34–37];

(c) Pro-RNA molecules could be synthesized in the prebiotic conditions of the atmosphere and the World Ocean of that time: ribose and nucleic acid bases could be synthesized from hydrogen cyanide (and its oligomers) and formaldehyde [38]. Amino acids as shown by Miller's experiments could be obtained from the mixture of ammonia, nitrogen, and hydrogen under electric discharge [39,40]. Lipid molecules could be formed by glycerol and fatty acids.

(d) Decisive factors for initial cells' origination might be the existence of a lipid membrane around the replicating pro-RNA molecule. This membrane would hinder the exit of products from the reaction mixture. Hargreaves and Deamer [41] proposed that the existence of lipid-like molecules was most important for biomembranes (two-dimensional liquids) to be formed. These lipid molecules would possess certain qualities: sphere formation through self-association, formation of water compartments inside the sphere, permeability for ions and water, elasticity and fluidity (self-reparation), etc. De Duve also stressed the importance of association of initial replicating molecules (pro-RNA) with membranous structures, "which could grow and divide themselves, and thus to be an object of real Darwin' selection" [42–45]. Determination of the process by which the nucleic acids could find themselves in the closed space of biomembrane has not been discussed in enough details.

The problem has been considered in the present work in connection with the findings that resulted from the study of interaction between double-stranded RNA and phospholipid vesicles [46].

1.2. Current knowledge on prokaryote-to-eukaryote transition

At present, there exists a number of hypothesis on the origin of eukaryotic cells from prokaryotic ones [47,48]. A number of possible schemes have been considered in the current concepts of the prokaryote-to-eukaryote transition. It was proposed that the eukaryotic nucleus arose from the fusion of two classes of proto-eukaryotic cells, namely eubacteria and archaeabacteria. The problem is considered here from a different viewpoint, in terms of the importance of the DNA–membrane interactions for this process [15]. Alongside the lipid–nucleic acid interactions [1,2,8,49], and (phospho)lipid–nucleic acid recognition [3,4], this type of fundamental interaction of the basic classes of biomacromolecules has lately received much attention from researchers concerned with the problems of nuclear matrix organization, regulation of gene expression [8], and gene transfer across biomembranes [10–12,50,51].

The phenomenon and the importance of the interaction of DNA with cellular membranous structures have been established for prokaryotes and eukaryotes. In these types of organisms, the DNA–membrane contacts provide sites for the initiation of transcription and replication processes, as well as regulating the expression of genome sites interacting with the inner nuclear membrane. The model for such contacts taking into account the role of DNA–lipid interactions has been discussed earlier [2,52].

According to this model, the prokaryotic Bayer's junctions (BJs) serving as the sites of DNA attachment to the membrane, act as the adhesion zones between the cellular wall and the cytoplasmatic membrane. The DNA–membrane contacts in eukaryotic cells appear to be the analogues of the nuclear pores or the porous annulate lamellae. This means excluding the concept of eukaryotic cells originating from one or another representative of the presently existing classes of bacteria with probable inclusion of chloroplasts or cyanobacteria into these cells at later stages. It is most likely that the ancestors of the present eukaryotes (the proto-eukaryotes) have ceased to exist. Indeed, if they do exist, then the state they have acquired in the process of evolution differs a great deal from that of their progenitors.

2. Hypothesis

2.1. Nucleic acid–lipid interactions in the origin of initial cells

The self-replicating pro-RNA molecule could find itself in the closed volume as the result of endocytosis or of some
other related process [45]. In the case of the more complex objects, lipid membrane polymorphic changes could play a key role in those processes how it was demonstrated for Ca$^{2+}$-induced capture of exogenic DNA by gram-negative bacteria [58]. To explain how the pro-RNA molecule could find itself in the closed membrane volume, we have to attract also our results of the study of the interaction between phospholipid vesicles and nucleic acids (plasmid DNA, RNA) in the presence of comparatively high concentration of metal (II) ions. Thus, it was shown [53–56] that the plasmid DNA condenses with phospholipid vesicles in the presence of Mg$^{2+}$ ions (from 15 to 50 mM), forming the genome precipitates during the centrifugation of the mixture. Such condensation does not take place at lower concentrations of Mg$^{2+}$ ions (0–10 mM). The same authors showed that the plasmid DNA in this complex is covered by a phospholipid bilayer, DNA being protected not only against enzymes (DNase, topoisomerase I), but also against dyes with fluorescent probes (ethidium bromide, DAPI), which intercalate into double-stranded DNA. The analogous phenomena have been demonstrated for DNA condensation with cationic lipids even in the absence of metal (II) ions. Taking into account the results of experiments mentioned above, it might be proposed that a self-replicating pro-RNA molecule could get inside a lipid membrane as a result of assembling in the presence of metal (II) ions, which were in abundance in those prebiotic conditions. Scheme 1 represents a chain of possible events in prebiotic period. There were the number of molecules of simple chemicals in prebiotic conditions, namely, nitrogen, hydrogen, carbon dioxide, hydrogen cyanide, existing in ocean at high temperature and electric discharges. Some lipidic molecules could be formed first at these conditions. It was reported, for example, that NASA scientists succeeded to form some vesicles from lipid material scraped from meteorites. Pre-RNA molecules could be processed then on the surface of those lipid bubbles-vesicles. Those pre-RNA could appear inside lipid vesicles at high concentration of divalent metal cations existing in excess at those conditions (Scheme 1) which could lead to the first self-reproductive systems.

### 2.2. The four proto-eukaryotic cell fusion model

One of the most natural concepts in this context is that of fusion of several proto-eukaryotic cells into a eukaryotic cell with a single nucleus [57]. The question of how it could happen has not been previously discussed. To gain a better insight into this process, it was thought worthwhile to consider the role of the nucleic acid–membrane contacts in the organization of the prokaryotic nucleoid. In this case, our target was the elucidation of the mechanism of the nucleus formation in the initial eukaryotic cell as well as the transition from the DNA–membrane contacts of proto-eukaryotes to the nuclear pores of eukaryotes. The basic requirements following from the cell fusion model suggest such orientation of the cells involved during their merging which excluded the problems for division of the ancestor cell, and the enormous advantages to metabolic events. The conditions must have been met during evolution as was evident from preparing hybrid eukaryotic cells in vitro.

Scheme 2 presents the scheme of formation of the eukaryotic cell from four different prokaryotic cells (Scheme 2a and b) [15]. The solid line displays the DNA (the prokaryotic cell’s nucleoid) attached at several points to the membrane. The fusion of four different cells is demonstrated by Scheme 2b (lateral view) and Scheme 2c (horizontal section). Scheme 2d shows the result of such fusion (top view). The dark spots on the membrane show the BIs becoming the DNA–MCs from the four former prokaryotic cells. There are four different sets of DNA–MC as shown by four types of lines. The light dotted line represents DNA on the further inner cell membrane. The double bacterial envelope (the cell wall and cytoplasmic membrane) is represented in Scheme 2d by the solid line.

For a better understanding of these stages of the process, we should assume that the integration of the nucleoids of
four proto-eukaryotic cells of different types to form one eukaryotic cell brings enormous advantages to such a hybrid cell, that is, a better chance for survival in a broader range of external conditions (nutrition, humidity, temperature range, atmospheric conditions). The result must not only be the survival of fortunate combinations of such hybrid cells, but also their flourishing, rapid growth and reproduction. Intensive synthesis of proteins, lipids and other bioactive molecules leads to the acceleration of the membranous structure's growth. Due to the high rigidity of the exterior membrane (the cell wall) in the hybrid and its remoteness from the cell centre, the growth is most probably made by the inner cytoplasmic membrane. However, the latter grows into the cell itself, with formation of protrusions, cisterns, cylinders, endoplasmic reticulum and other organelles.

The availability of the two closely located membrane layers as well as the DNA in the cell may lead, according to our concept, to formation of the structures analogous to the porous plates and nuclear pores. These DNA–MCs can be gradually transformed from the BJ onto the structures of the porous plates type. This inevitably results in increased numbers of the DNA–MC, hence the enhanced expression of the hybrid cells genome, which may give the cells an additional chance for survival. Having reached the highest density of pores in the endoplasmic reticulum (ER), after the DNA condensation in the course of mitosis, the ER is likely
to start to disintegrate, transforming into a set of membrane vesicles (Scheme 2c). In the prophase of the cell cycle, a precursor of the nuclear envelope may be formed from these vesicles around the chromosomes (the former nucleoids not shown in Scheme 2).

The likelihood of confluence of four different cell types is exceedingly low, and the probability of the hybrid cell’s survival is even less. Consequently, the evolution of eukaryotes from prokaryotes took place over a long period of time, perhaps billions of years. It appears that the most prolonged period was that of selection of optimal nucleoid combinations, whereas the transformation of the BJs and the DNA–MCs into the nuclear pores in a fortunate daughter cell may have materialized rapidly. The cell walls of prokaryotes may have formed a common cytoplasmic membrane of eukaryotes, which could serve as the basis for the formation of other cellular membranes. Mitochondria and chloroplasts have probably retained the ancient type of the DNA–MC as well as relative autonomy in the cell, paving the way for their later inclusion into the eukaryotic cell.

On the other hand, many publications have been recently summarized in the context of knowledge about the nature of the universal tree of living organisms [57,58]. There are some doubts about the existence of proof for the prokaryote-to-eukaryote transition in an evolution-like manner. Until now, data have been able to support in general the existence of phylogenetic relationships among the three kingdoms (domains), namely the Bacteria (eubacteria), Archaea (archaeabacteria) and Eucarya (eukaryotes) as the parts of a new tripartite view of life, having probably as a common root, the cenancestor [58].

3. Conclusions

(1) Self-replicating pro-RNA molecules could be synthesized on the surface of lipid bubbles, and could get inside lipid membrane as a result of pro-RNA-lipid assembling in the presence of metal (II) ions, which were in abundance in those prebiotic conditions.

(2) The possible mechanism is proposed for the formation of eukaryotic cell from four different proto-eukaryotic cells. The fusion of the nucleoids of four proto-eukaryotic cells of different types to form one eukaryotic cell could bring enormous advantages to such hybrid cell, that is, a better chance for survival in a broader range of external conditions. Intensive synthesis of proteins, lipids and other bioactive molecules could lead to the acceleration of the membranous structure’s growth.

(3) It appears that the most prolonged period (maybe millions years) could be that of selection of optimal nucleoid combinations, whereas the transformation of the BJs and the DNA–MCs to the nuclear pores in a fortunate daughter cell could materialize more rapidly.

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