THE EVOLUTION OF PREBIOLOGICAL SELF-ORGANIZATION: PROBABLE COLLOID-CHEMICAL EVOLUTION OF FIRST PROKARYOTIC CELLS

V.Liebl, V.J.A. Novák, Z. Masinovský,
*B.Pacltová, L.Bejšovcová

Department of Evolutionary Biology, Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Praha 4, and *Department of Paleontology, Faculty of Sciences, Charles University, Albertov 6, 128 43 Praha 2, Czechoslovakia

Abstract. This is an attempt to analyse the mechanisms of self-assembly in the course of the origin and early evolution of life on the Earth. A special attention is paid to the investigation of transient stages between the physico-chemical and biological bases of self-assembly, including experimental models and paleontological results. The theory of coacervate-in-coacervate is discussed from the point of view of evolution of first procaryotic cells. Many of the high developed structures of the contemporary cells, such as ribosomes, chromosomes, lipid membranes, some other organelles etc., are claimed to posses a rudimentary polyionic coacervate character.

Theoretical considerations, observations as well as experiments demonstrate that life is a result of a complex evolution of the matter in the Universe and on the Earth,where long-term suitable conditions were available /42,43,3,56,13,6,4,2,50,53/. Selfassembly of atoms, molecules, supermolecular structures, etc., is one of the fundamental principles of the origin and existence of living organisms /3,19,8,32,52,13,26,14,58/. The study of phylogenesis of the recent organisms and of their communities since the origin of life reveals the evolution of the basic mechanisms of the selfassembly, which manifests itself in a continuous increase in their complexity /13,44,36,52,31,29, 29a/ and specificity.

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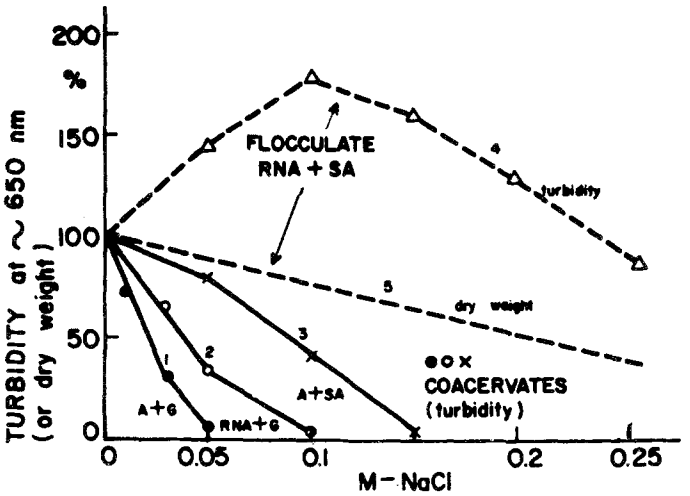
The specificity of the chemical self-assembly, e.g. the self-sequencing of amino acids into thermal polyamino acids i.e. proteinoids /13/ is determined by three factors: 1. Different stability of aminoacids at the conditions of the thermal synthesis. 2. Their mutual transformations during this process. 3. The selective mutual affinity of amino acids, which is determined by their structure, its own stereoelectronic identity and reactivity /29/.

The next step of the evolution of the self-assembly mechanisms was the colloid-chemical organization of proteinoids and of other macromolecules /or association colloids, e.g. phospholipids/ into individual phase-separated systems: coacervates /1,42,21,59,67,44/, microspheres /13,62/, marigranules /66/, lipid vesicles, i.e. liposomes /15,61,18/, etc. All these multimolecular phases have their own internal specific medium, separated from the external medium. They exchange components and energy with the surroundings and they behave thus thermodynamically as open systems /32,7/.

Coacervates represent highly concentrated specific lyophilic dynamically cross-linked sols /special colloidal solutions/ which can be transformed even in a rigid gel. Coacervates can have the form of droplets /microcoacervate/ or they may fuse, i.e. coalesce into a coacervate layer /macrocoacervate /1/. Mechanisms of the colloid-chemical formation of coacervates and microspheres are similar. Microspheres /type of microcoacervate/ however, are mostly formed from abiotically synthesized proteinoids, which represents a great succes of S.W. Fox and his school /13,12,11/ from the point of view of abiogenic origin of life. Coacervates are mostly prepared from biopolymers isolated from contemporary high evolved organisms, however, they can be also formed from synthetic polymers, proteinoids, etc. /1,54,40,41/.

The complex, i.e. polyionic coacervation, which seems to have played the main role in the origin of multimolecular prebiotic protocellular systems /42,9,60,21, 25,59,39,44,41/ depends on: 1. The presence of oppositely charged polyions, mainly highly hydrated macromolecules, independent of their intramolecular structure. 2. The properties of liquid, mainly aqueous medium, such as pH, ionic strength, temperature, etc. /1,42,40,24,26,20/.

Our conception of the evolution of primordial cells, the coacervate-in-coacervate theory /38,39/ originated from Oparin´s coacervate theory /42,43,44/, Fox´s proteinoids and microspheres conception /12,13,10,11,14,1/,



Influence of salts (e.g. NaCl) on complex colloid systems formed by polyionic interactions

The interaction of the macromolecular components was characterized by turbidity measurement or by dry weight of sediments. The turbidity (measured with a red filter) and also the dry weight of sediments in the absence of salts was taken as 100%.

The concentration of macromolecular components was 0.02% and the maxima of turbidity (mostly near pH 3.5) from turbidity--pH curves are shown in the Figure.

1. Arabinate A + gelatine G (1:1) coacervate
2. Ribonucleic acid RNA + gelatine G (1:1) coacervate
3. Arabinate A + serumalbumine SA (1:1) coacervate
   (The turbidity as well as dry weights of sediments of all these coacervates decrease with increasing salt concentration i.e. with ionic strength. The A+SA coacervate is the most stable.)

4., 5. Ribonucleic acid RNA + serumalbumine SA (3:7) form flocculate instead of coacervate (flocculate formed from these compounds in 1:1 proportion has turbidity optimum at pH 2.5). The turbidity (curve 4) of flocculate increases with increasing salt concentration up to about 0.1 M, the dry weight of sediment, i.e. centrifuged flocculate, decreasing continuously (curve 5). Smaller particles of the flocculate, sedimenting slowly, are formed with increasing NaCl concentration.*)

The decrease of the sediment dry weight is given by increasing solubility of the coacervate or flocculate with higher ionic strength. The flocculates are shown to be more stable in salt solutions than coacervates.

*) The smaller the particles, the more they scatter light - hence the increase in the turbidity.

and some other studies. This theory of evolution of first prokaryotic cells is further based on following experimental findings: 1. On the properties of coacervate droplets which mainly coalesce together, form colonies and layer, sediment to the bottom, and practically do not divide. If coacervates and similar structures "divide" then it is the physico-chemical process, different from biological cell division generally governed by chromosome, i.e. nucleic acid replication and function. 2. Easy formation of complex coacervates even from very diluted solutions of polyionic polymers, e.g. proteins, proteinoids, polynucleotides, polysacharides, phospholipids, etc. 3. The coacervates ability to absorb selectively various substances, especially some polymers such as proteins /enzymes/, proteinoids, polynucleotides, phospholipids, etc., from the aquenous medium /9,25/. 4. The ability to split, transform, regulate or synthesize different, especially macromolecular compounds in the specific internal coacervate medium, with some catalyzer, as in an open system /42,9, 60,24,26/. 5. The model similarities between coacervates and highly evolved structures of contemporary cells, like some parts of protoplasm, ribosomes, chromosomes, "liquid" protein-phospholipid membranes, etc. /1,42,63,21,20, 26,60/. 6. The feasibility of preparation and coexistence of composite coacervate drops /1/, e.g. secondary nucleoprotein coacervates /mainly droplets/ in primary glycoprotein coacervate droplets or sedimented coalesced layer /23,25/.

Fig. 1. demonstrate a completed scheme of our coacervate-in-coacervate theory /38,39,34,37,25,26,,36/.

a/ Some droplets of a primary e.g. proteinoid coacervates or microspheres /formed in "prebiotic soup" in primordial water basins, by evaporation, cooling, polyionic interactions, etc./, tend to fuse /coalesce/ and form a colonies and layer of primary coacervates, i.e. macrocoacervate primordial slime /25/.

b/ The synthesis of polynucleotides /~~~/ under probable protoenzymic and matrical action of proteinoids took place /17,24,30,45/ in the nutrient, catalytical and protective /against UV-radiation, chemical decomposition, etc. 27,41/ primary coacervate layer, rather than in coacervate droplets. The primordial slime could have been mixed with clay minerals, etc., which could also catalyze formation of both monomers and especially of polymers such as polyamino acids, polynucleotides, etc. /49,5,65,2/.

c/ Initial replication of single-stranded polynucleotides /~~~/ i.e. primordial genome and formation of double-

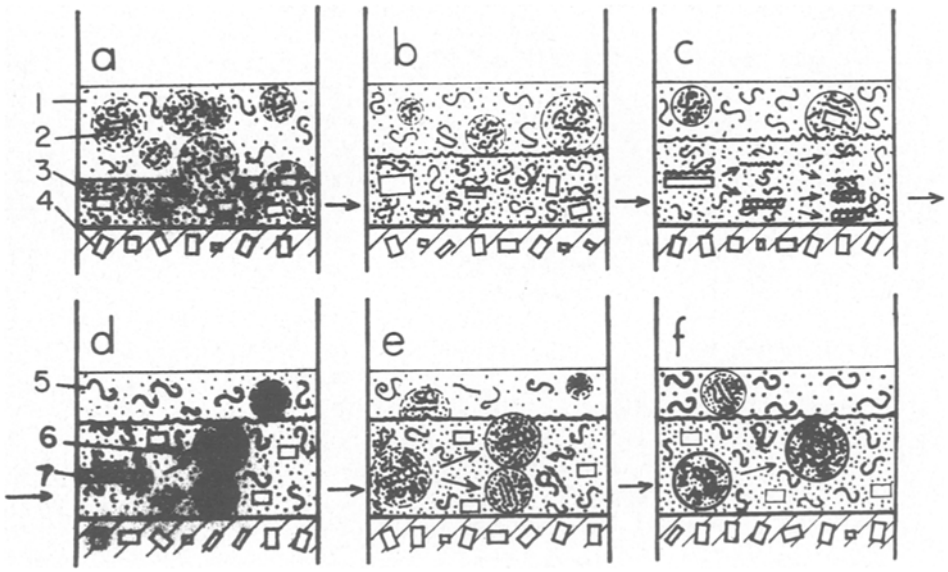


Fig. 1. Probable evolution of primordial prokaryotic cells from secondary nucleoprotein coacervates or microspheres, in the primary proteinoidal coacervate layer /coacervate-in-coacervate theory/. The completed scheme.
1-prebiotic soup with proteinoids /~/ and other compounds and structures.
2-primary proteinoid coacervate droplets or microspheres.
3-primary proteinoid coacervate layer, i.e. primordial slime with clays, etc.
4-clay minerals, geological inorganic bottom.
5-molecules of proteinoids and other protobiopolymers.
6-secondary nucleoprotein coacervate droplet or microsphere.
7-primordial replicating double-stranded nucleic acid molecule /RNA or DNA/.

-stranded polynucleotides catalyzed by proteinoids mainly in primordial slime.

d/ The primitive genome /probably similar to some primordial virus, 35/ evolved the genetically directed synthesis of more and more specific acidic and basic protoproteins, some with enzymic activities, which probably occured on protoribosomes formed as a kind of nucleoprotoprotein coacervate structure. These coded protoproteins assembled with protogenom, some other nucleic acids, proteinoids, polysacharide-polyanions, protoribosomes, etc., forming secondary replicating nucleoprotein coacervate droplets or microspheres,

probably with some rudimental membrane. This is the coacervate-in-coacervate stage.

e/ Covering of secondary coacervate droplets by membranes continued in nutrient and protective primordial slime. Semipermeable envelopes stabilized these protocells chemically and mechanically. The coacervate-like protocytoplasm could be then further differentiated, mainly by the action of basic proteins which bound polyionically preferably some nucleic acids /22/, giving less viscous protocytosol /with smaller and smaller dynamical crosslinking coacervate relations/. Much more complex protoribosomes of supercoacervate type /23/ with biospecific hydrogen bound and other interactions between RNAs and mainly basic proteins evolved. Genome /nuclear region/ its replication, transcription and translation i.e. protein synthesis developed, see /12,10,16/; metabolic pathways and regulations, cell division - as biological processes directed by genome - have been established.

f/ Evolution of: cell wall, prokaryotic circular genome, prokaryotic metabolism, prokaryotic cell cycle and prokaryotic cell.

In this way the primordial i.e. first prokaryotic cell probably gradually evolved. Some of its components preserved some of the characters of the original secondary polyionic 'sol-gel' coacervate, the "coacervate relicts", e.g. the cytoplasm; others continued to develope in this direction, forming more complicated structures on the coacervate principle within the new medium - the "supercoacervates", e.g. ribosomes, chromosomes, phospholipid membranes, etc. /23,26,20/.

Experimental verifications of this conception of macromolecular and colloid-chemical evolution of the primordial prokaryotic cell is quite difficult, but in some stages it is possible. a/ We can prepare coexisting secondary nucleoprotein coacervates inside primary glycoprotein coacervates /generally so-called composite coacervates /1,23,25/. The two are well visible in optical microscope, especially after suitable staining. b/ In the presence of phospholipids and similar compounds coacervates are generally covered by membranes /1,60/; on the surface of microspheres membranes were observed /13/. c/ We have prepared coacervate-like nucleoprotein model system from ribosomal RNA + basic proteins + Mg^{2+}, of colloid-chemical character similar to hypothetical protoribosomes /26,12,10/. d/ Some suitable thermally synthesized proteinoids usually have polyfunctional very

low catalytic, i.e. protoenzymic activities /55,13,23/, being able to catalyze various protobiochemical reactions. We studied phosphatases-like reactions, redox--reactions /28/ and some synthesizing reactions. Jungck and Fox /17/ synthesized some oligonucleotides from ATP by proteinoid microspheres. We tried to synthesize oligomers from ADP using proteinoids as protoenzymes /24/. The synthesizing effectivity of proteinoids is generally very low but still appreciable /Fig. 2/.

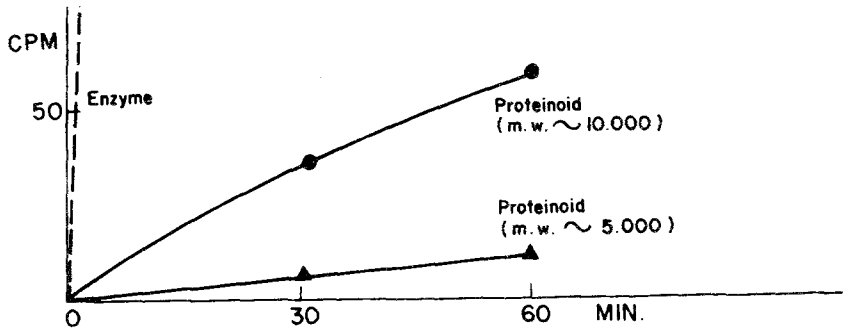
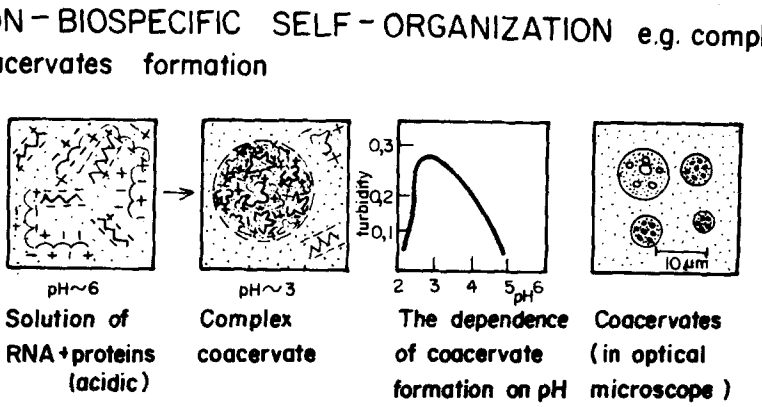
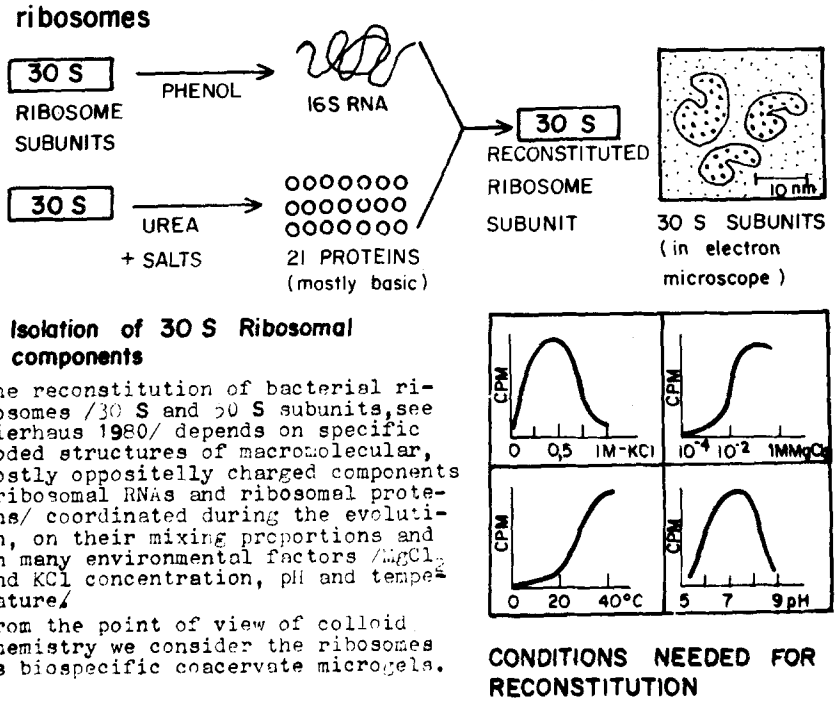


Fig. 2. Polymerization of radioactive adenosine-diphosphate /^{3}H-ADP/ catalyzed by proteinoid purified by gel filtration. CPM indicate the radioactivity on paper chromatograms /amount of the synthesized polymer/. The fraction of proteinoid of higher molecular weight has higher synthesizing activity. For comparison, very diluted bacterial enzyme polynucleotide-phosphorylase, shows much higher /about 4 orders/ activity.

The above mentioned "constructionistic" experiments, modelling the processes of prebiological self-organization, when compared with the data on the biological self--assembly, allow us to approach the understanding of the mechanism of the latter and its formation during the evolution. Let us try to show it using as an example ribosomes - specialized nucleoprotein microparticles /the size about 15 nm/, which realize the protein synthesis in contemporary cells. The analysis of the state, composition and the character of binding at ribosomes allows, in our oppinion, to consider this particles to be gelous structures of a coacervate type /Fig. 3/. The highly specific organization of the ribosomal components determined by the genetic code /proteins and ribonucleic acids/ is based on the qualitatively higher biospecific bonds: hydrogenic, hydrophobic, etc.; but the basic structure of ribosomes in normal, i.e. non-halophilic bacteria /20/, seems to us to be generally determined by polyionic in-



The complex i.e. polyionic coacervation /or flocculation/ depends on the mixing proportions of the oppositelly charged components /from which almost one must be macromolecular or associative colloid/; thus, it also depends on pH in the medium; it occurs in environment with low ionic strength.



The reconstitution of bacterial ribosomes /30 S and 50 S subunits, see Nierhaus 1980/ depends on specific coded structures of macromolecular, mostly oppositelly charged components /ribosomal RNAs and ribosomal proteins/ coordinated during the evolution, on their mixing proportions and on many environmental factors /$MgCl_2$ and KCl concentration, pH and temperature/.

From the point of view of colloid chemistry we consider the ribosomes as biospecific coacervate microgels.

Fig. 3. The comparison of non-biospecific and biospecific self-organization.

teractions typical for the complex coacervate, which could be realized already in prebiological self-assembly /26/. This suggestion is in agreement with the results of /12,10/, who studied the self-assembly and the functional activity of so-called protoribosomes, which are formed on the basis of polyionic, non-biospecific interactions between the synthetic polyribonucleotides and the basic proteinoids, modulated to a certain extent by the affinity between some nucleotides and amino acids. An analogous, but high biospecific self-assembly, reconstitution of ribosomes, can take place between the components of bacterial ribosomes /64,33/.

The conception suggesting the existence of the chemical and prebiological forms of self-assembly, which preceded the origin of the living systems, is supported also by the paleontology. Paleontological record of the morphologically definable microfossils from the oldest Precambrian terrestrial sediments /about $3,5.10^9$ years/ support the idea which considers the prebiotic system as a transient stage between the chemical and biological evolution on the Earth /57,51,47,46/. On the other hand in the surface samples of the silicites of the Upper Proterozoic, contaminations of subrecent organic residues were demonstrated /48/. From the contemporary knowledge follows the necesity to investigate methodically all Precambrian finds of microfossils and their value from the point of view of the origin and evolution of life in the Precambrian. The question whether the oldest "microfossils" represent real living primordial organisms, capable of replication, or some prebiotic systems only, cannot be decided with certainty yet.

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