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Achievements and Challenges in Generating Protocell Models

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Dedicated to Pier Luigi Luisi on the occasion of his 70th birthday.

The most promising approach for the synthesis of a cell-mimicking chemical system that can undergo Darwinian evolution is the coupling of a self-replicating ribozyme with a self-reproducing lipid vesicle.[1] The polymerisation of RNA and the in situ formation of fatty acid (FA) vesicles was first combined in the 1990s in Luisi's group, albeit with the help of an enzyme, polynucleotide phosphorylase (PNPase).[2] In a similar approach, Joyce, Deamer and co-workers encapsulated PNPase in phospholipid vesicles that could be permeated by the substrate of PNPase, ADP, by adjusting the temperature to the main phase transition temperature (T_m) of the lipid bilayer.[3] Permeability again takes center stage in a recent study by Szostak and coworkers that employs mixtures of simple lipids to prepare model protocells. They developed a system that was able to use template-directed synthesis to generate a genetic polymer within these model protocells—this time, however, without using an enzyme.[4] Protocells are hypothetical precursor structures that are assumed to have preceded the first cells during the prebiological chemical evolution and eventually led to the first biological cells.[5]

The Szostak lab has done a formidable job in recent years in working towards the creation of a potentially prebiotic protocell model. Their earlier work includes studies on the growth and division of FA vesicles,[6] the encapsulation of RNA adsorbed on clay-particles,[7] hammerhead ribozyme activity in stabilized FA-based vesicles,[8] the selective growth of osmotically challenged vesicles,[9] and the diastereoselective permeability for ribose.[10]

While big challenges remain, and not all of the above-mentioned processes work under the same conditions, the recent paper by Mansy et al.[4] brings us a step further on the way towards a laboratory simulation of the origin of life. Mansy et al.[4] managed to render mixed FA-based vesicles sufficiently permeable for activated nucleotides but stable enough so that a DNA-oligomer, which served as the template, remained encapsulated inside the vesicle.

This new report consists of three parts. First, the authors studied the permeability of mixed FA vesicles for ribose, examining factors like surface charge, chain length, unsaturation and branching. They found that farnesol, a highly branched and unsaturated isoprenoid, has the strongest effect on permeability in a 1:2 mixture with myristoleic (C14:1) acid; this is most likely caused by the creation of packing disorders. In the second part, the permeability of nucleotides was examined. As expected, permeability was reduced as the number of charges increased (AMP vs. ADP vs. ATP), but enhanced by complexation with Mg^{2+} and by activation as imidazolide. Finally, the system developed by Mansy et al.[4] was able to carry out a template-controlled, nonenzymatic synthesis of an oligonucleotide within vesicular compartments that were composed of chemically simple, potentially prebiotic amphiphilic lipids: decanoic acid, decanol and glycerol monodecanoate (4:1:1).[11] Because activated nucleotide monomers were added to the vesicles already containing the template, the permeability properties of the vesicles used were the key point for the success of the work;[12] the nucleotides had to move across the vesicle membranes without leakage of the encapsulated macromolecular templates.

Protocell research is where biophysics, synthetic chemistry, molecular biology, surfactant self-assembly and nanotechnology meet—in a field at the border between chemistry and biology and strongly driven by theoretical considerations and computer simulations.[13] This is due to both the complexity of contemporary cells and the different ideas about what theoretically constitutes a minimal cell. Early cells were certainly structurally and functionally simpler than modern ones, although they already possessed a considerable degree of organization. It is obvious that compartmentalization must have been an important property of the first cells, but compartmentalization alone was not sufficient. The first cells probably contained a primitive metabolism linked to a template-based replication system, which allowed informational molecules to be copied. These were then linked to the metabolic activity and to the formation of amphiphilic molecules, which constituted the compartments boundary, to allow for compartment growth and reproduction.[14–16]

One of the challenges in the field of protocell research today is the preparation of a chemical system which fulfils the conceptual requirements set for early cells—at best with molecules which are potentially prebiotic (see Figure 1). To reach this goal, a number of experimental problems still have to be solved. Some of these are 1) linked to the molecular structures of the components of which the protocells were possibly made of (for example, prebiotically relevant lipids, templates, catalysts), and 2) linked to analytical and systemic methodologies to investigate those pro-

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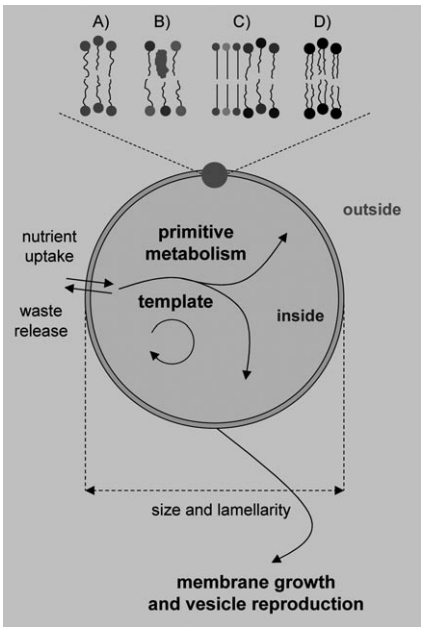

Figure 1. Schematic representation of the cross section of a spherical unilamellar vesicle, which serves as model of a protocellular system. The vesicle shell, which separates the inside from the outside, is composed of amphiphiles with varied chemical structure; some examples are A) mixtures of single chain lipids, B) mixtures of single chain lipids and hydrophobic non-amphiphilic compounds, C) mixtures of lipids which do not mix homogeneously, but assemble into domains with different molecular order and D) double chain lipids. The protocell hosts a template that is copied and a template-dependent primitive metabolism, which results in the formation of membrane compounds that allow for its growth and eventually for vesicle reproduction. The vesicle permeability properties, which are given by the membrane composition and the chemical composition inside and outside of the vesicles, control the (selective) nutrient uptake and waste release. The size and lamellarity of the vesicles are in turn controlled by the membrane composition and by the physicochemical conditions during the vesicle formation process.

tocell models and their dynamic behaviour. This latter point comprises exchange of components between outside and inside (and *vice versa*), chemical transformations and protocell morphology changes.

With respect to a further increase in the complexity of a protocell model, there is still a need for new methods that allow quantification of vesicle bilayer permeability. The methodology used by Mansy et al.[4] largely depends on an indirect assay using fluorescent probe molecules.[17] The method is based on the self-quenching of an entrapped fluorophore that becomes diluted upon solute permeation, leading to an increase in fluorescence intensity. Despite the great progress that has been made during the last decades in the field of lipid vesicles as biomembrane models and as drug delivery systems,[18] which is essential for protocell research at large, direct vesicle permeability measurements still remain a challenge.

From a strictly prebiotic point of view, there are a number of chemical questions that need to be addressed. First, the number of presumably prebiotic amphiphiles which form bilayers is rather limited today: FAs and fatty alcohols, alkylphosphates and phosphonates,[19] polyprenylphosphates.[20] The range of experimental conditions under which they form stable membranes (for example, change in pH, salt content) is also limited.[21,22] Second, even though the lipid mixtures used by Mansy et al.[4] are an improvement with respect to earlier systems that only used single types of FAs, more work will certainly have to be devoted to membrane and vesicle reproduction processes.[23,24] Finally, it is important to recall that the template, the primer and the (activated) nucleotides used by Mansy et al.[4] are chemically complex, *nota bene* optically active, and it will be a big challenge to 1) either convincingly demonstrate that a prebiotic synthesis of nucleotides and oligonucleotides is possible, or 2) to find alternative, chemically simpler molecular systems with similar properties.[25]

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