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## Origin of Life: Protocells Red in Tooth and Claw

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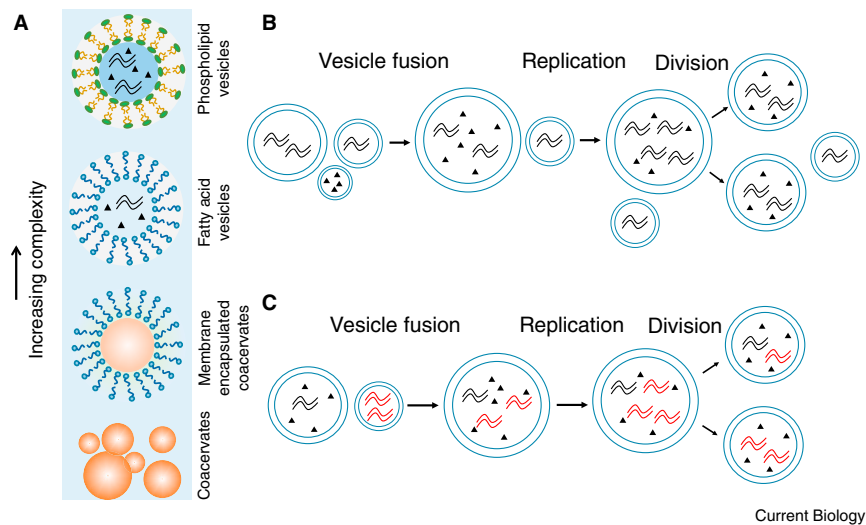
To study the origin of life, synthetic biologists construct simple ‘protocells’, but previous models were not able to reproduce both genome and membrane sustainably. A recent advance feeds the protocells by vesicle fusion, suggesting a practical pathway for indefinite self-reproduction.

What is life, and how can we make it? NASA’s Exobiology Program uses the working definition of life as “a self-sustaining chemical system capable of Darwinian evolution” [1]. Several research labs have undertaken the task of synthesizing an organism that meets this definition. It seems clear that some propagating genetic information is necessary, whether it is a self-replicating RNA [2] or a system of enzymes and DNA [3]. In addition, there are convincing arguments for encapsulating the genetic system inside self-replicating vesicles,

creating a primitive entity called a ‘protocell’. Compartmentalization enables a crowded, cell-like interior with concentrated contents [4] and is essential for combatting the evolution of parasitic genomes that replicate without contributing to the protocell’s metabolism [5,6]. In 2011, PCR amplification of encapsulated DNA was successfully combined with the growth and division of phospholipid-based vesicles [7]. However, the vesicle composition in that system changed after self-reproduction, because the

added membrane lipids differed from the original membrane composition, causing the membrane composition to become progressively distorted. The system, therefore, failed to meet the criterion of ‘self-sustaining’ in NASA’s definition of life. Now, the same group has overcome this problem by fusing the self-reproducing vesicles with feeder vesicles, thus allowing the vesicle composition to be sustained over multiple generations [8].

The idea of protocells can be traced back to the Russian biochemist



**Figure 1. Evolution of protocells.**

(A) A scheme for evolution of protocells from coacervates. (B) In the competition among protocells, the ‘rich get richer’ as the larger vesicle grows more quickly and fuses with the feeder vesicles. (C) In a virus-like strategy, a parasitic genome (red) lurks within a small vesicle and awaits fusion. Once inside an actively metabolizing protocell, the parasitic genome replicates rapidly and overtakes the host’s genome (black).

Alexander Oparin, who suggested that the spontaneous concentration of oppositely charged polyelectrolytes into liquid droplets forming a separate phase from water (coacervates) could be the first living compartments (Figure 1A) [9]. Coacervate droplets are attractive for synthetic systems due to the simplicity of their components and have been investigated as a protocell model [10]. For example, protein can be expressed using a cell-free system within the molecularly crowded matrix of a polysaccharide/polypeptide coacervate [11]. However, the absence of an enclosing membrane causes these systems to be quite permeable, allowing nucleic acids to diffuse between different droplets and rendering the formation of genetically distinct individuals difficult [12]. On the other hand, coacervates may serve as structural templates for more advanced protocells with membranes, as fatty acids spontaneously self-assemble into continuous membranes at the surface of preformed coacervate droplets (Figure 1A) [13]. Such a system would transition naturally into another major experimental model of protocells, nucleic acids (particularly self-replicating or non-enzymatically replicating RNA) encapsulated in fatty acid membranes.

These membrane-bound protocells have many advantages in addition to the ability to retain genetic material, including the prebiotic plausibility of their components and the observation of Darwinian competition between protocells [14]. The substantial progress on developing such protocells was recently reviewed by Blaine and Szostak [15].

While protocells using fatty acids are attractive for several reasons (and they seem likely to someday fulfill the NASA definition of life), they are, like coacervates, a transitional form along the path toward a more robust form of life. Fatty acid vesicles, being negatively charged, are notably sensitive to ionic conditions and particularly to divalent cations (e.g.,  $Mg^{2+}$ ) that are often required for folding and function of nucleic acids [16]. Interestingly, just as coacervates may lead to fatty acid protocells, fatty acid protocells may evolve into more stable phospholipid protocells, as any mechanism that produced diacyl lipids would promote growth of those vesicles (Figure 1A) [17]. Picking up from that transition point, the ‘semi-synthetic’ approach to a protocell drops the emphasis on prebiotic plausibility and instead borrows a

minimal set of molecular components from modern biology to create a highly simplified cell. For example, a water-in-oil emulsion containing Q $\beta$  replicase (an enzyme that replicates the Q $\beta$  phage genome) has been used to investigate evolutionary dynamics [18]. Alternatively, protocells can be constructed using phospholipid membranes, which are quite robust to ions such as  $Mg^{2+}$  (Figure 1A). Phospholipid vesicles (liposomes) can encapsulate nucleic acids, ribosomes, proteins, and low-molecular weight compounds (amino acids, nucleotides, etc). Interestingly, solute encapsulation during liposome formation may yield encapsulated concentrations that are higher than expected by random chance [19].

One of the prominent protocell models based on phospholipid membranes is studied by Sugawara and colleagues, who previously encapsulated a DNA genome with DNA polymerase in phospholipid vesicles, allowing amplification by PCR. To grow the membrane, a cationic lipid precursor was added, which could be converted into a cationic membrane lipid by a catalyst embedded in the phospholipid membrane [7]. A fascinating observation was that the frequency of liposome division was notably higher when PCR-amplified DNA was encapsulated within the vesicle. It appeared that the cationic membrane component attracted the anionic DNA to the inner leaflet of the lipid bilayer. This caused accumulation of cationic lipid around DNA, resulting in a mass imbalance between the leaflets and consequent division of the vesicle through membrane budding. This type of electrostatic interaction may be fairly general, as the association of cationic peptides with zwitterionic or anionic membranes can attract RNA molecules to the membrane [20]. However, self-reproduction of the phospholipid vesicle system was previously limited, because although the original vesicles were composed of phospholipids, there was no way to introduce additional phospholipids. Therefore, the membrane composition became increasingly dominated by the (non-phospholipid) cationic lipid, which could not form

stable vesicles on its own. In addition, there was no way to add more nucleotides and enzyme. Ultimately, a mechanism to supply phospholipids and nucleotides to the protocells was required.

The solution to this problem has recently been reported by the same group, through delivery of nucleotides, enzyme and phospholipids by fusion of ‘feeder’ vesicles to the protocells [8]. The system consists of two kinds of vesicles. The target vesicle (simulated newborn protocell) contains the DNA genome, PCR primers, and polymerase, encapsulated in a phospholipid membrane with the cationic lipid. However, these target vesicles lack dNTPs. On the other hand, the conveyor vesicles contain dNTPs encapsulated in a phospholipid membrane (without cationic lipid). The positively charged target vesicles fuse with the negatively charged conveyor vesicles at low pH. The authors describe four phases of a primitive cell cycle. In the ingestion phase, a low pH environment induces vesicle fusion, effectively transporting needed substrates (dNTPs and phospholipids) to the protocells. In principle, other reagents (e.g., DNA polymerase) could also be replenished at this step with appropriate formulation of the conveyor vesicles. Second, in the replication phase, the environment is neutralized and the DNA is amplified by PCR. Third, during the maturation phase, the amplified DNA localizes the cationic membrane molecules to the inner leaflet of the membrane, which apparently forms a complex with the amphiphilic membrane catalyst to produce more cationic lipids. Finally, the division phase of the protocell starts after enrichment of a high local concentration of cationic lipids leads to a budding deformation that divides the vesicle into two daughter protocells.

Kurihara *et al.* [8] thus enact a new, extremely heterotrophic approach to feed protocells. Instead of taking up nutrients through the membrane, which is subject to the permeability properties of the membrane, the protocells simply fuse to other vesicles. These protocell cannibals thus gain direct access to their victim’s contents. Interestingly, larger vesicles amplify DNA more quickly,

presumably because they contain more DNA relative to the membrane. As DNA amplification is tied to membrane growth, this suggests that the ‘rich get richer’ as larger vesicles grow and divide more quickly (Figure 1B). This competition based on a biophysical phenomenon echoes an earlier finding that increased osmotic pressure (e.g., resulting from genome replication) causes some vesicles to ‘steal’ membrane from other vesicles [14]. Vesicle cannibalism raises the stakes of the competition between protocells — inactive protocells do not merely fail to grow, they are actually eaten by others.

Feeding through fusion also opens the door to a pageant of evolutionary phenomena. Strategies to preferentially sequester resources (e.g., enzymes or membrane catalysts) during division or rapidly produce or acquire anionic mass could evolve. In addition, the conveyor vesicles need not be devoid of a genome. Fusion of vesicles containing different genomes would create intracellular competition between unrelated genomes, and could lead to genetic novelty through recombination. Parasitic genomes could lurk within conveyor vesicles, awaiting fusion to a target vesicle susceptible to takeover (Figure 1C). Although these protocells are rather advanced compared to the prebiotic milieu, further study of this evolvable system promises to be a rewarding endeavor.

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