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Preview

Synthetic chemistry recreates transitional forms in prebiotic membrane evolution

Ranajay Saha[1] and Irene A. Chen[1,*]

Membranes are an essential part of living systems. Today's phospholipid membranes are believed to have originated as spontaneously self-assembled fatty acid membranes billions of years ago. How might the transition to phospholipids occur? In this issue of *Chem*, Pulletikurti et al. recreate transitional forms based on prebiotic chemical reactions, filling in "missing links" of early membrane evolution.

Cell membranes were essential for creating individual entities encapsulating molecular cooperatives (protocells) during the early evolution of life.[1] The cell membranes of today are composed primarily of phospholipids, which are built upon a glycerol backbone linked to two fatty acid "tails" and a modified phosphate "head" group. Phospholipids self-assemble to form highly stable membranes, but the molecules are produced by biosynthetic machinery and do not appear to be prebiotically plausible.[2] On the other hand, fatty acids by themselves are also able to self-assemble into membrane vesicles in the right chemical environment. Indeed, fatty acids can be produced in various prebiotic syntheses and are found in carbonaceous chondrite meteorites thought to represent the chemical composition of early Earth.[3,4] Fatty acid vesicles are a prominent experimental model for protocells given their semi-permeable nature enabling the encapsulation of genetic material, such as RNA, in addition to a fascinating capability for spontaneous growth and division through multiple mechanisms.[5] However, compared with phospholipids, fatty acid vesicles are limited by their sensitivity to the chemical environment, especially instability at non-neutral pH or in the presence of significant divalent cation concentrations (e.g., Mg^{+2}), which are often important for RNA function.[6,7] At some point, presumably quite early in evolution, these prebiotic but unstable fatty acid membranes would have had to transition to the phospholipid membranes of today. How might such a transition have occurred?

In evolutionary biology, the fossil record provides evidence of transitions through so-called "transitional forms," which possess a combination of characteristic traits from both an ancestral species and a modern species and thus illustrate a phylogenetic relationship. A well-known example of a transitional form is the 150-million-year-old *Archaeopteryx*, whose fossils indicate the presence of both dinosaur traits (e.g., teeth and a bony tail) and bird traits (e.g., feathered wings and a wishbone), supporting the dinosaurian ancestry of modern birds (Figure 1A).[8] Although there is little chance of discovering protocell fossils (which would have to be nearly 4 billion years old given the estimated age of the last universal common ancestor), synthetic chemists can recreate plausible transitional forms to study their properties and assess the plausibility of chemical evolution via such forms.

A proposed transition from fatty acids to phospholipids proceeds via two intermediate forms. First, cyclic phospholipids (e.g., cPC10; Figure 1B) can be produced from fatty acids and glycerol in a one-pot phosphorylation by diamidophosphate (DAP).[9] Cyclic phospholipids have a single fatty chain (like fatty acids) and a cyclic glycerol-phosphate head group (resembling phospholipids). Next, hydrolytic ring opening of the head group followed by esterification with fatty acids could produce a second transitional form, diacyl-phospholipids, which have two fatty tails (e.g., GDDP; Figure 1B) and a glycerol-phosphate head group. Later modification of the head group would yield modern-day phospholipids.

In this issue of *Chem*, Pulletikurti et al. show that the two transitional forms indeed constitute stable membranes, establishing a plausible pathway for prebiotic membrane development from fatty acids to phospholipids.[10] Interestingly, mixed-composition vesicles consisting of the transitional cyclo-phospholipids and their precursors (fatty acids and their glycerol monoesters) exhibited enhanced properties compared with those of the pure membrane compositions. For example, a mixture of cPC10 and the glycerol monoester (GMD) exhibited greater stability than either pure system given that GMD alone does not form stable vesicles and cPC10 alone forms vesicles only at relatively high concentrations. Similarly, mixed-composition vesicles

[1]Department of Chemical and Biomolecular Engineering, Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA 90095, USA

*Correspondence: ireneachen@ucla.edu

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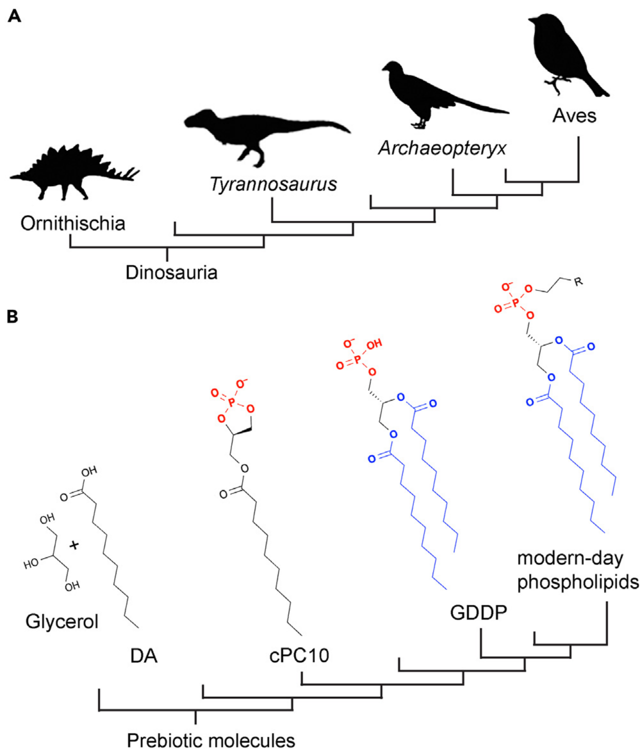

Figure 1. Illustrations of transitional forms in animal evolution and the proposed prebiotic membrane evolution

(A) *Archaeopteryx* and *Tyrannosaurus* are among the transitional forms found in the fossil record characterizing the evolution of modern birds (Aves) from dinosaurs. Adapted from Ritchison.[8]

(B) Cyclic phospholipids, such as cPC10, are produced from fatty acids (e.g., decanoic acid [DA]) and glycerol with phosphorylation with DAP. The hydrolysis of the cyclic phosphate moiety followed by esterification leads to diacyl-phospholipids (GDDPs), whose modification would produce modern phospholipids.

containing the next transitional form, diacyl-phospholipids with a simple glycerol-phosphate head group, also showed enhanced stability. GDDP alone formed vesicles at low concentrations relative to those of cPC10-based vesicles, consistent with the additional stability granted by the two fatty tails. However, GDDP-only vesicles were restricted to a narrow pH range and low metal-ion concentration as a result of the charged phosphate moiety, and a mixture of GDDP and a precursor (GMD) increased stability by both measures.

Moreover, the overall progression of cPC10-based to GDDP-based vesicles gave increasing added advantages in terms of the ease of formation and stability with respect to variations in pH, temperature, and metal-ion concentration, indicating selectable traits. Indeed, Pulletikurti et al. also show that the greatest jump in vesicle stability would occur at the last transitional step: modification of the head group of GDDP-like molecules to produce modern-day phospholipids. If membrane composition were influenced by protocellular RNAs, such as ribozymes, then Darwinian evolution could favor increasingly sophisticated membranes, in addition to any physical or chemical separation processes operating on the vesicles. This study therefore neatly addresses the dual questions of *how* fatty acids could transition to phospholipids (by demonstrating vesicles made of the intermediate forms) and *why* the transition would be advantageous for protocell fitness (increased stability and robustness to chemical environments).

Proposals for prebiotic scenarios are often contested on the basis of geochemical plausibility. However, synthetic chemistry and synthetic biology allow experimenters to test concrete hypotheses and determine environmental limits on specific scenarios. Along these lines, Pulletikurti et al. simulated a prebiotic reaction starting from glycerol, fatty acids, DAP, and imidazole. Wet-dry cycling to promote esterification, phosphorylation, and hydrolysis led to a crude mixture of lipids that indeed formed into vesicles. This work exemplifies the synthetic approach to understanding the origin of life. Although it might not be possible to definitively prove the historical events leading to the specific origin of life on Earth, researchers can address the broader question of outlining the mechanisms, reactions, and pathways by which living systems and their essential components, such as membranes, could have emerged and evolved.

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AUTHOR CONTRIBUTIONS

R.S. and I.A.C. wrote the paper.

DECLARATION OF INTERESTS

I.A.C. is a co-founder of Paralos Bioscience Inc.

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