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Chemistry and Physics of Primitive Membranes

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Abstract A membrane boundary structure was essential for the advent of cellular life. The membranes of contemporary cells are composed of a mosaic of proteins embedded in a bimolecular layer of phospholipids, each of which requires a complex enzymatic pathway for its synthesis. The earliest forms of life could not have had such a highly evolved pathway in place. Amphiphilic monocarboxylic acids are present in carbonaceous meteorites and can be synthesized under simulated geochemical conditions. Such compounds have physical and chemical properties that allow them to assemble into bilayer membranes and are therefore plausible components of the first cellular membranes.

Keywords Artificial cells · Encapsulation · Lipid vesicles · Membranes

1 Introduction

Life on the Earth most likely arose from vast numbers of natural experiments in which various combinations of organic molecules were mixed and recombined to form complex interacting systems, then exposed to sources of energy such as light, heat, and oxidation-reduction potentials presented by donors and acceptors of electrons. This mixing and recombination probably did not occur in free solution, but rather in fluctuating environments at aqueous–mineral interfaces exposed to the atmosphere under conditions that would tend to concentrate the organic material so that reactions could occur. Through this process, incremental chemical evolution took place over a period of ten to several hundred million years after the Earth had cooled sufficiently for water vapor to condense into oceans. At some point, membrane-bounded systems of molecules appeared that could grow and reproduce by using energy and nutrients from the environment. An observer seeing this end product would conclude that such systems were alive but would be unable to pinpoint the exact time when the complex structures took on the property of life.

Here we will assume that the structures described above would be recognizable cells. The first cellular life had four key properties: (1) polymeric materials were encapsulated within a membrane-bounded structure; (2) the bounded system of molecules had the ability to capture energy and nutrients from the local environment; (3) the system could grow by spontaneous noncovalent addition of components from the environment and by catalyzed energy-dependent formation of covalent bonds between monomers to form polymers; and (4) the growing system could reproduce and evolve using a process directed by a replicating information-storage molecule.

Current research efforts have progressed to the point where the above processes have been investigated individually, so that the challenge now is to assemble them into an integrated system that exhibits the properties of the living state. This chapter focuses on the self-organizing properties of amphiphilic compounds that produce microscopic compartments necessary for the appearance of the first cellular forms of life.

2
Self-Assembly Process in Early Forms of Life

All cellular life today incorporates two processes we will refer to as self-assembly and directed assembly (Fig. 1). The latter involves the formation of covalent bonds by energy-dependent synthetic reactions and requires that a coded sequence in one type of polymer in some way direct the sequence of monomer addition in a second polymeric species. On the other hand, spontaneous self-assembly occurs when certain compounds associate through noncovalent hydrogen bonds, electrostatic forces, and nonpolar interactions that stabilize orderly arrangements of small and large molecules. Three well-known examples include the self-assembly of water molecules into ice, DNA

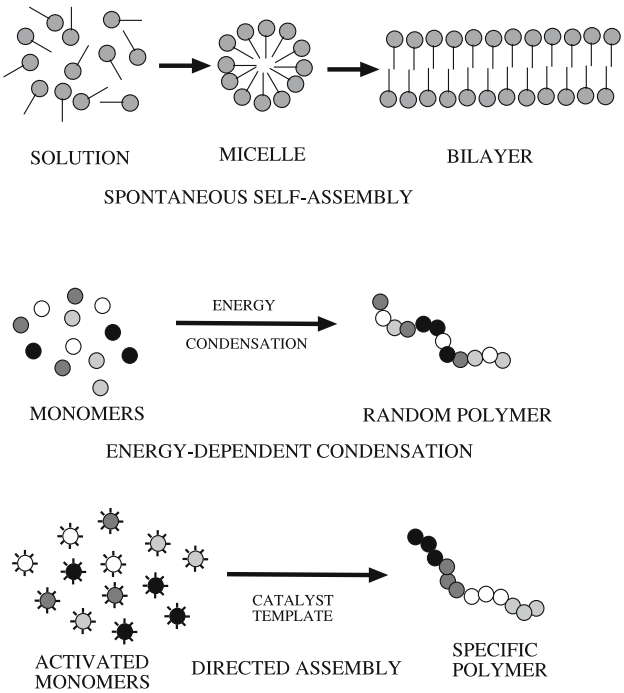


Fig. 1 Cellular life today uses both self-assembly and directed assembly processes to grow. Self-assembly (*upper diagram*) is essential to the synthesis and stability of membrane structures and protein folding, while directed assembly (*lower diagram*) underlies the synthesis of proteins according to the base sequences in DNA and mRNA. We assume that on the early Earth, random polymers similar to peptides and nucleic acids were produced by a yet unknown synthetic pathway (*center*). The random polymers, if capable of growth in a membrane-bounded microenvironment, would have been subjected to selection and thereby begin biological evolution

strands into a double helix, and newly synthesized protein chains into functional folded conformations. The latter two examples occur spontaneously, but the processes are enzyme mediated for regulatory reasons or to exclude undesirable conformations. A fourth self-assembly process involves certain compounds that can form closed membrane-bounded microenvironments. Such boundary structures, and the compartments they produce, have the potential to make energy available in the form of ion gradients and can provide a selective inward transport of nutrients. Furthermore, membranous compartments in principle are capable of containing unique systems of macromolecules. If a yet unknown macromolecular replicating system of polymers could be encapsulated within a membrane-bounded compartment, the components of the system would share the same microenvironment, and the result would be a major step toward cellularity, speciation, and true cellular function [1–6].

We know very little about how this event might have occurred at the origin of cellular life, but recent advances have provided clues about possible sources of amphiphilic molecules, assembly of membrane structures, and encapsulation mechanisms by which large molecules can be captured in membrane-bounded microenvironments. Here we will describe the chemical and physical properties of such systems and several experimental models that incorporate certain properties related to the origin of cellular life.

3
Sources of Amphiphilic Compounds on the Early Earth

There are only two possible sources of organic compounds on a primitive planetary surface: delivery during late accretion in the late Hadean era, followed by chemical evolution, or synthesis by geochemical processes in the primitive atmosphere and hydrosphere. Earlier investigations focused on chemical synthesis of monomers common to the primary macromolecules involved in living systems, with the goal of determining whether it was possible that biologically relevant compounds were available on the primitive Earth [7, 8]. Most of these studies emphasized water-soluble compounds such as amino acids, nucleobases, and simple carbohydrates. Here we will focus on self-assembling hydrocarbon derivatives. The most straightforward geochemical synthesis of hydrocarbons and their amphiphilic derivatives is the Fischer–Tropsch type synthesis (FTT). In this reaction, carbon monoxide is mixed with hydrogen and exposed to a hot catalyst such as metallic iron. Under these conditions, a remarkable reaction occurs in which hydrocarbon chains are synthesized by single-carbon additions, yielding alkanes, monocarboxylic acids, and alcohols ranging up to 30 or so carbons in length. Examples of such syntheses described in the literature include the pioneering

observations of Oró et al. [10], with more recent results reported by McCollum et al. [11] and Rushdi et al. [12].

In the classic experiments of Miller and Urey [7, 8] the mixture of reduced gases was assumed to be a simulation of the original terrestrial atmosphere, which, by analogy with the outer planets, would have contained hydrogen, methane, ammonia, and water vapor. At sufficiently high energy fluxes, such mixtures of reduced gases generate hydrogen cyanide and formaldehyde, which in turn react by Strecker synthesis to produce amino acids, purines, and a variety of simple sugars. The proposal that organic compounds could be synthesized under prebiotic conditions was given additional weight when it was convincingly shown that carbonaceous meteorites contained amino acids, hydrocarbons, and even traces of purines [13–15]. If such meteorites represent samples of the primitive solar system components that underwent synthetic chemical reactions, it was reasonable to assume that similar reactions may have occurred on the Earth's surface.

This view was challenged in the late 1970s when lines of evidence emerged that the early atmosphere was composed of carbon dioxide and nitrogen rather than the mixture of reducing gases assumed by the Miller–Urey model [16, 17]. Carbon dioxide does not support synthetic pathways leading to chemical monomers [9], so interest was drawn to the second potential source of organic material: extraterrestrial infall in the form of micrometeorites and comets. This scenario was first proposed by Oró [18] and Delsemme [19] and more recently extended by Anders [20] and Chyba and Sagan [21]. The total organic carbon added by extraterrestrial infall over $\sim 10^8$ years of late accretion can be estimated to be in the rage of 10^{16}–10^{18} kg, which is several orders of magnitude greater than the total organic carbon in the biosphere. From such calculations it seems reasonable that extraterrestrial infall was a significant source of organic carbon in the prebiotic environment [22].

The discovery of biologically relevant compounds in meteorites also indicated that organic synthesis can occur in other environments, which immediately leads to the question of sources and synthetic pathways. Clues to a possible source of the meteoritic organics have been provided by infrared and millimeter astronomy. Vibrational and rotational spectral features obtained from molecular clouds indicate the presence of a plethora of carbon-containing compounds [23, 24]. Spectral features obtained from molecular clouds indicate the presence of a hundred or more carbon-containing compounds [23, 24]. Because dense molecular clouds are the birthplace of stars and solar systems, it seems reasonable that the organic substances present in comets and the parent bodies of meteorites were derived from the carbon compounds present in the original molecular cloud that gave rise to the solar system.

Dense molecular clouds attenuate the interstellar radiation field, permitting the synthesis and survival of more complex species in the gas phase than

is possible in the diffuse interstellar medium. At the low temperatures in these dark molecular clouds (10–50 K), mixtures of molecules condense to form ice mantles on the surfaces of refractory dust grains where they can participate in additional gas-grain chemical reactions. Comparison of infrared spectra of low temperature laboratory ices with absorption spectra of molecular clouds indicates that interstellar ices are mainly composed of H_2O mixed with CO, CO_2, CH_3OH, NH_3, and other components, the latter ingredients generally comprising 5 to 15% of the total. The ices are exposed to ionizing radiation in the form of cosmic rays (and secondary radiation generated by their interaction with matter) and UV photons impinging upon the attenuated diffuse interstellar medium (ISM) or generated by stars forming within the cloud.

Laboratory experiments have shown that radiation processing of simulated presolar ices leads to more complex molecular species [25–27]. Hundreds of new compounds are synthesized, although the starting ices contain only a few simple common interstellar molecules. Many of the compounds formed in these experiments are also present in meteorites and cometary and asteroidal dust (interplanetary dust particles – IDPs), and some are presumably relevant to the origin of life, including amino acids [28, 29], quinines [30], and amphiphilic material [31].

The consensus view is that organic molecules and their building blocks are synthesized in dense molecular clouds and then become components of the presolar nebula where they are further altered; these nebula give rise to stars, solar systems, and the parent bodies of meteorites. However, the molecules must be delivered to habitable planetary surfaces if they are to take part in the origin of life. This requires that they survive the transition from the dense cloud into a protostellar nebula and subsequent incorporation into planetesimals, followed by delivery to a planetary surface. Theoretical calculations suggest that a fraction of the extraterrestrial organics present in comets should survive even during impact with a planetary atmosphere [32], and experimental results confirm that some organic species do, in fact, survive planetary accretion. The most convincing evidence comes from deuterium isotopic measurements of meteorites and interplanetary dust particles (IDPs) collected on Earth. Such objects contain many of the same compounds and classes of compounds produced in interstellar simulations, and meteoritic organics frequently have large deuterium excesses [33]. These excesses are difficult to understand in terms of solar system chemistry but may be explained by a variety of interstellar chemical processes that produce organic compounds [34].

Even today, meteorites and IDPs deliver organic materials to the Earth's surface at a rate of $\sim 1 \times 10^6$ kg/year [35]. During the late bombardment period, which lasted until about 4 billion years ago, the amount of extraterrestrial organic material brought to the prebiotic Earth was likely to have been orders of magnitude greater [21]. Thus, the early Earth must have been

seeded with organic matter created in the interstellar medium, protosolar nebula, and asteroidal/cometary parent bodies.

From these considerations we conclude that both exogenous delivery and endogenous synthetic pathways provided organic material to the prebiotic environment, a process summarized in Fig. 2. We cannot be certain about the relative amounts of endogenous synthesis and extraterrestrial delivery of organics, but it is likely that both sources played significant roles in the subsequent emergence of life by providing specific molecular species that were essential ingredients. The question to be answered now is what fraction was degraded to simple carbon compounds such as CO and CO_2 and what fraction was incorporated directly into the molecular systems leading to the origin of life.

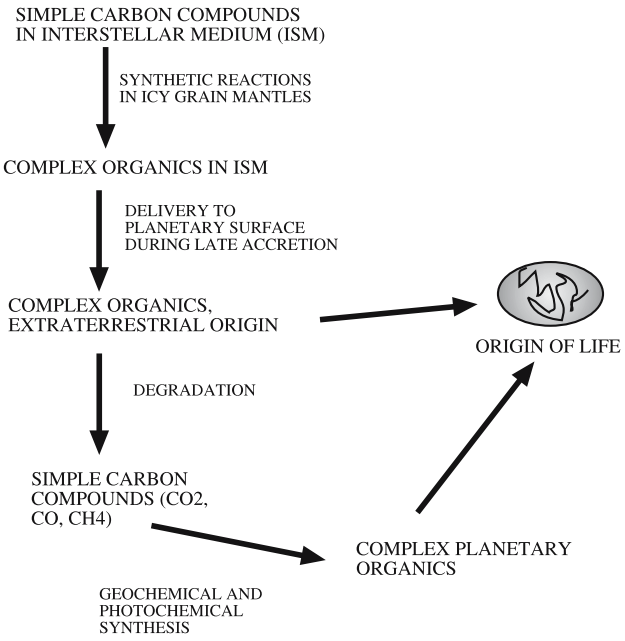


Fig. 2 All terrestrial carbon was initially delivered to the primitive Earth during accretion. Much of the carbon was degraded to simple carbon compounds that could then undergo synthetic geochemical reactions to produce more-complex species. However, a fraction of the delivered organic carbon was likely to survive intact, especially during late accretion. This fraction had the potential to be incorporated into the molecular systems that gave rise to the origin of life

4
What Amphiphiles Composed the First Cell Membranes?

Amphiphilic molecules are among the simplest of life's molecular components and are readily synthesized by nonbiological processes. Virtually any normal alkane having ten or more carbons in its chain takes on amphiphilic properties if one end of the molecule incorporates a polar or ionic group (see below). The simplest common amphiphiles are therefore molecules such as monocarboxylic acids (anions), monoamines (cations), and alcohols (neutral polar groups).

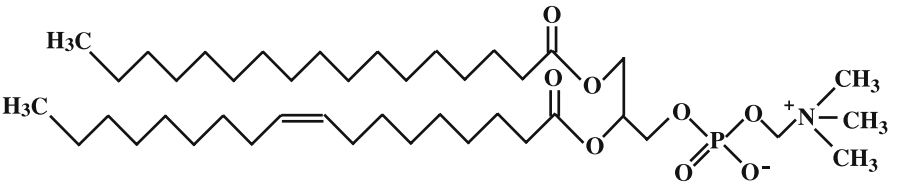
$$CH_3 - (CH_2)_n - COOH \rightarrow H^+ + CH_3 - (CH_2)_n - COO^- \quad \text{(anion)}$$

$$CH_3 - (CH_2)_n - NH_2 + H^+ \rightarrow CH_3 - (CH_2)_n - NH_3^+ \quad \text{(cation)}$$

$$CH_3 - (CH_2)_n - OH \quad \text{(neutral amphiphile)}$$

Lipids are far more diverse chemically than other typical biomolecules such as amino acids, carbohydrates, and nucleotides. The definition of lipids includes simple fatty acids and their glycerol esters, sterols such as cholesterol, and phospholipids, sphingolipids, and cerebrosides. Lipids are generally defined by their common hydrophobic character, which makes them soluble in organic solvents such as chloroform. Virtually all lipids also have a hydrophilic group, which makes them surface active.

Eukaryotic phospholipids typically have two fatty acid chains linked to a glycerol by ester bonds, with the third position of the glycerol esterified to a phosphate group. Most phospholipids also have a head group such as choline, ethanolamine, or serine attached to the phosphate, and one such lipid is shown below (1-palmitoyl, 2-oleoyl phosphatidylcholine). The precise function of the variable head groups has not yet been established.



Scheme 1

The other lipid commonly present in eukaryotic membranes is cholesterol, a polycyclic structure produced from isoprene by a complex biosynthetic pathway. It is interesting to ask whether it is conceivable that prebiotically plausible reactions might also produce complex amphiphiles. The earliest investigations aiming to answer this question were carried out by Hargreaves et al. [36], Oró and coworkers [37, 38], and, more recently, Ourisson et al. [39] and Conde–Frieboes and Blochliger [40]. In all such reactions,

energy-dependent condensation reactions are used to produce complex lipids from mixtures of phosphate, fatty acid, and glycerol. Examples of such lipids include phosphatidic acid and phosphatidylcholine, both of which readily self-assemble into membranous vesicles.

Although it is clear that complex lipids can be synthesized under laboratory simulations using pure reagents, the list of required ingredients does not seem plausible under prebiotic conditions. Therefore, it is unlikely that early membranes were composed of complex lipids such as phospholipids and cholesterol. Instead, there must have been a source of simpler amphiphilic molecules capable of self-assembly into membranes. One possibility is lipid-like fatty acids and fatty alcohols, which are products of FTT simulations of prebiotic geochemistry [12] and are also present in carbonaceous meteorites. Furthermore, as will be discussed later, these compounds form reasonably stable lipid bilayer membranes by self-assembly from mixtures (Fig. 4a).

5 The Fluid Mosaic Model of Membrane Structure: Relation to Early Membranes

In the 1970s, the fluid mosaic concept emerged as the most plausible model to account for the known structure and properties of biological membranes [41]. The fact that membranes exist as two-dimensional fluids (liquid disordered) rather than in a gel state (solid ordered) was clearly demonstrated by Frye and Ededin [42], who showed that the lipid and protein components of two separate membranes diffuse into each other when two different cells were fused. Since that time, numerous studies have measured the diffusion coefficient of lipids and proteins in membranes, and the diffusion rates were found to correspond to those expected of a fluid with the viscosity of olive oil rather than a gel phase resembling wax.

Because the lipid components of membranes must be in a fluid state to function as membranes in living cells, it is reasonable to assume that primitive membranes in the first forms of cellular life must also have had this property. Straight-chain hydrocarbons have relatively high melting points due to the ease with which van der Waals interactions can occur along the chains. Any discontinuity in the chains interrupts these interactions and markedly decreases the melting point. As an example, stearic acid contains 18 carbons in its alkane chain and melts at 68 °C, while oleic acid, with a cis-double bond between carbons 9 and 10, has a melting point near 14 °C. If cellular life today requires fluid membranes, it is reasonable to assume that the earliest cell membranes were also composed of amphiphilic molecules in a fluid state.

The idea that the proteins of biological membranes are embedded in a fluid sea of lipids arose from our increasing understanding of membrane struc-

ture. It has been demonstrated in numerous ways that most of the proteins associated with membranes are embedded in the lipid bilayer phase, rather than simply adhering to the surface. As a general rule, membrane proteins have stretches of hydrophobic amino acids in their sequences, and these are threaded back and forth through the bilayer multiple times, thereby anchoring the protein to the membrane. The hydrophobic proteins often are involved in production of pores, or transmembrane channels, that are essential for ion and nutrient transport processes.

Could similar channels be produced in the bilayer membranes of primitive cells? There is no doubt that channel-like defects appear when a nonpolar peptide interacts with a lipid bilayer. For instance, polyleucine or polyalanine has been induced to fuse with planar lipid membranes, and the bilayers exhibited transient bursts of proton conductance [43]. Surprisingly, channel-like conductance also appears when RNA is selected for its ability to bind to phospholipids [44]. From these observations it is fair to say that if random polymers were being produced by some unknown synthetic reaction on the early Earth, some of those polymers were likely to have been able to penetrate bilayer membranes and produce channels that bypassed the permeability barrier. This is an area that is ripe for further investigations, as described in a recent review by Pohorille et al. [45].

6
Function of Membranes in Early Cells

Membranes have many functions in addition to acting as a container for the macromolecular polymers of life. Three primary membrane functions associated with a protocell would include selective inward transport of nutrients from the environment, capture of the energy available in light or oxidation-reduction potentials, and coupling of that energy to some form of energy currency such as ATP in order to drive polymer synthesis (Fig. 3).

The simplest of these functions is that of a permeability barrier that limits free diffusion of solutes between the cytoplasm and external environment. Although such barriers are essential for cellular life to exist, there must also be a mechanism by which selective permeation allows specific solutes to cross the membrane. In contemporary cells, such processes are carried out by transmembrane proteins that act as channels and transporters. Examples include the proteins that facilitate the transport of glucose and amino acids into the cell, channels that allow potassium and sodium ions to permeate the membrane, and active transport of ions by enzymes that use ATP as an energy source.

It seems unlikely that the first living cellular systems had time to evolve highly specialized membrane transport systems, which brings up the ques-

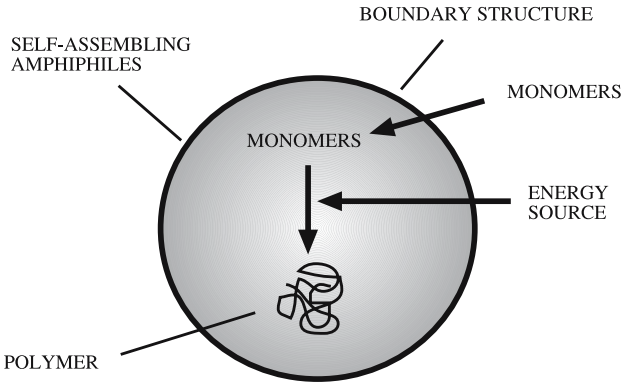


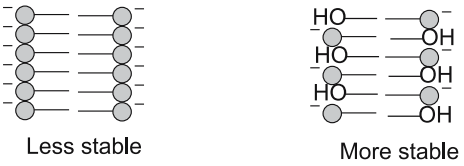
Fig. 3 A protocell would have had a minimal set of functional properties, including self-assembly of boundary membranes, transport of monomers, and capture of energy to drive polymerization reactions, and encapsulation of polymer systems capable of growth

tion of how early cells overcame the membrane permeability barrier. One possibility is that simple diffusion across the bilayer may have been sufficient. To give a perspective on permeability and transport rates by diffusion, we can compare the fluxes of relatively permeable and relatively impermeable solutes across contemporary lipid bilayers. The measured permeability of lipid bilayers to small, uncharged molecules such as water, oxygen, and carbon dioxide is greater than the permeability to ions by a factor of $\sim 10^9$. For instance, the permeability coefficient of water is approximately 10^{-3} cm/s, and the permeability coefficient of potassium ions is 10^{-11} cm/s. These values mean little by themselves, but make more sense when put in the context of time required for exchange across a bilayer. Measurements show that half the water in a liposome exchanges in milliseconds, while potassium ions have half-times of exchange measured in days.

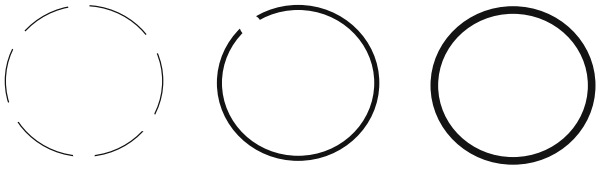
We can now consider some typical nutrient solutes like amino acids and phosphate. Such molecules are ionized, which means that they would not readily cross the permeability barrier of a lipid bilayer. Permeability coefficients of liposome membranes to phosphate and amino acids have been determined [46] and were found to be in the range of 10^{-11}–10^{-12} cm/s, similar to ionic solutes such as sodium and chloride ions. From these figures one can estimate that if a primitive microorganism depended on passive transport of phosphate across a lipid bilayer composed of a typical phospholipid, it would require several years to accumulate phosphate sufficient to double its DNA content or pass through one cell cycle. In contrast, a modern bacterial cell can reproduce in as short a time as 20 min.

If bilayers are so impermeable to solutes like amino acids and phosphate, how could primitive cells have had access to these essential nutrients? One clue may be that modern lipids are highly evolved products of several billion years of evolution and typically contain hydrocarbon chains 16 to 18 carbons

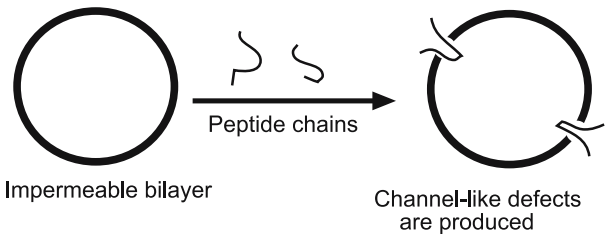
in length. These chains provide an interior "oily" portion of the lipid bilayer that represents a nearly impermeable barrier to the free diffusion of ions such as sodium and potassium. The reason is related to the common observation that "oil and water don't mix," That is, ion permeation of the hydrophobic portion of a lipid bilayer faces a very high energy barrier called Born energy, which is associated with the difference in energy for an ion in a high dielectric medium (water with a dielectric constant of 80) compared to the same ion in a low dielectric medium (hydrocarbon with a dielectric constant of 2). This energy barrier is immense, up to 40 kcal/mole [47].



A. Influence of charge, hydrogen bonding on stability



B. Influence of bilayer thickness on stability



C. Channel-like defects induced by peptide chains

Fig. 4 Stability and permeability of self-assembled amphiphilic structures. Amphiphilic molecules such as fatty acids having carbon chain lengths of 9 or more carbons form bilayer membranes when sufficiently concentrated. **a** Pure bilayers of ionized fatty acid are relatively unstable but become markedly more stable as long chain alcohols are added. **b** Dimensions of the amphiphile also play a role. Shorter chain amphiphiles (9–10 carbons) are less able to form bilayers, while those of intermediate chain length (12–14 carbons) produce stable bilayers that also are permeable to ionic and polar solutes. Longer chain lengths (16–18 carbons) produce bilayers that are increasingly less permeable to solutes [48]

However, recent studies have shown that permeability is strongly dependent on chain length [48]. For instance, shortening phospholipid chains from 18 to 14 carbons increases permeability to ions by a thousandfold (Fig. 4b). The reason is that thinner membranes have increasing numbers of transient defects that open and close on nanosecond time scales, so that ionic solutes can get from one side of the membrane to the other without dissolving in the oily interior phase of the bilayer. Ionic solutes even as large as ATP can diffuse across a bilayer composed of dimyristoylphosphatidylcholine, a 14-carbon phospholipid [49]. On the early Earth, shorter hydrocarbon chains would have been much more common than longer chain amphiphiles, suggesting that the first cell membranes were sufficiently leaky so that ionic and polar nutrients could enter while still maintaining larger polymeric molecules in the encapsulated volume. However, it should be kept in mind that polymeric compounds may also have been present in the prebiotic environment. If these happened to interact with the lipid bilayer of a membrane compartment, channel-like defects could have been produced that permitted transport of polar and ionic nutrients (Fig. 4c).

7 Growth Processes in Protocells

Earlier reports [50] showed that vesicles composed of oleic acid can grow and "reproduce" as oleoyl anhydride spontaneously hydrolyzed in the reaction mixture, thereby adding additional amphiphilic components (oleic acid) to the vesicle membranes. This approach has recently been extended by Hanczyc et al. [51], who prepared myristoleic acid membranes under defined conditions of pH, temperature, and ionic strength. The process by which the vesicles formed from micellar solutions required several hours, apparently with a rate-limiting step related to the assembly of "nuclei" of bilayer structures. However, if a mineral surface in the form of clay particles was present, the surface in some way catalyzed vesicle formation, reducing the time required from hours to a few minutes. The clay particles were spontaneously encapsulated in the vesicles. The authors further found that RNA bound to the clay was encapsulated as well.

In a second series of experiments, Hanczyc et al. [52] found that the myristoleic acid vesicles could be induced to grow by addition of fatty acid to the medium, presumably by incorporating fatty acid molecules into the membrane, rather than by fusion of vesicles. If the resulting suspension of large vesicles was then filtered through a polycarbonate filter having pores 0.2 μm in diameter, the larger vesicles underwent a kind of shear-induced division to produce smaller vesicles. This process could be repeated several times (Fig. 5).

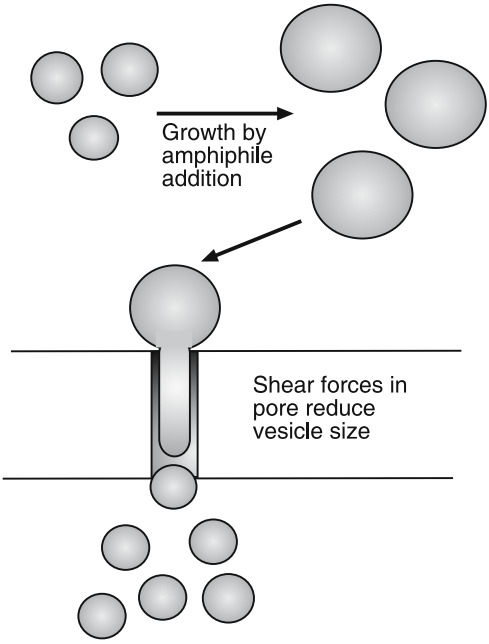


Fig. 5 Fatty acid vesicles can grow by addition of fatty acid molecules to the membrane and are then dispersed into smaller vesicles by passage through a porous filter. The cycle of growth and dispersion was repeated several times and presumably could go on indefinitely [52]

This remarkable series of experiments clearly demonstrated the relative simplicity of producing a complex system of lipid, genetic material, and mineral catalyst in a model protocellular structure that can undergo a form of growth and division.

8
Encapsulation Mechanisms

Even if membranous vesicles were commonplace on the early Earth and had sufficient permeability to permit nutrient transport to occur, these structures would be virtually impermeable to larger polymeric molecules that were necessarily incorporated into molecular systems on the pathway to cellular life. The encapsulation of macromolecules in lipid vesicles has been demonstrated by hydration–dehydration cycles that simulate an evaporating lagoon [53] or by freeze–thaw cycles [54]. Molecules as large as DNA can be captured by such processes. For instance, when a dispersion of DNA and fatty acid vesicles is dried, the vesicles fuse to form a multilamellar sandwich structure with

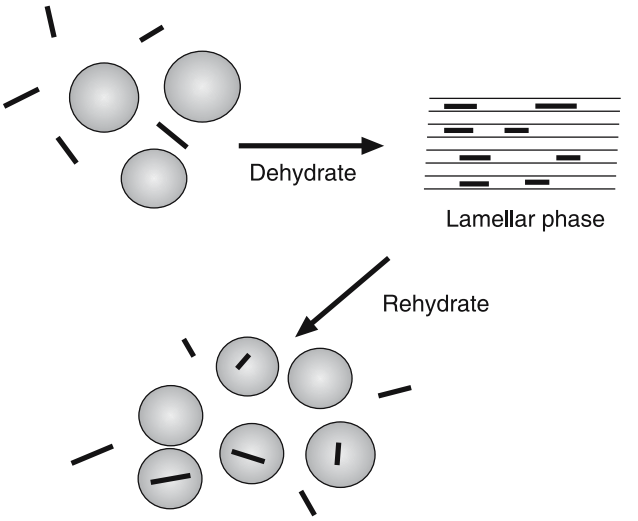


Fig. 6 Macromolecules are readily encapsulated in lipid vesicles in a single cycle of dehydration–hydration [53]. Such wetting–drying cycles would have commonly occurred in the prebiotic environment at intertidal zones

DNA trapped between the layers. Upon rehydration, vesicles reform that contain highly concentrated DNA, a process that can be visualized by staining with a fluorescent dye (Fig. 6). Several enzymes have also been encapsulated using similar procedures [55].

9 Could Mineral-Water Interfaces Act as Precursors to Life?

Given that organic compounds are made available on a planetary surface as described above, they must then be organized into mixtures that are sufficiently concentrated to undergo chemical reactions. It seems likely that most of the compounds would be delivered to extensive early oceans, rather than the relatively small area of available volcanic terrain, but this immediately leads to a conundrum: global concentrations of organic material in the seas would have been too dilute to undergo synthetic chemical reactions [56]. For this reason, it has been suggested that mineral/aqueous interfaces were primary agents in concentrating and organizing organic solutes, and perhaps catalyzing specific reactions related to life processes [57, 58]. Corliss et al. [59] and Baross and Hoffman [60] first proposed that life began as an organic film on mineral surfaces in subsurface geothermally active sites. Such films would provide a microenvironment of low water activity so that hydrolytic back

reactions would not continuously degrade more complex molecules such as polymers formed by condensation reactions. As an energy source, either dissolved hydrogen gas or the mineral surface itself would provide a source of reducing power [61–63]. In this scenario, membrane encapsulation and a system of information transfer would evolve at some later time.

As a specific chemical example of a mineral-dependent reaction pathway, Huber and Wächtershäuser [64] described an experimental model in which a slurry of nickel and iron sulfide was found to promote the formation of acetic acid from carbon monoxide and methyl mercaptan (CH_3SH). Peptide bond formation could also be demonstrated [65]. These conditions were considered to represent a simulation of a primordial geothermal system in which metal sulfides at high temperatures ($\sim 100\,^{\circ}C$) provide a reaction pathway for the initial steps of an autotrophic metabolism. This is an interesting result that is pertinent to the synthesis of organic material and confirms earlier observations that a variety of free energy sources can drive the formation of simple organic molecules. In the case of a mineral surface, the chemical potential is contained in the reactants and the mineral itself, rather than an impinging energy source such as electrical discharge or UV photons.

Martin and Russell [66] have taken this concept a step further. They note that certain iron sulfide minerals contain microscopic pores in the size range of cells ($\sim 10–100\,\mu m$) and propose that such cavities could provide a mineral version of a membranous boundary structure. The authors suggest that the cavities may be able to concentrate nutrient organic solutes that could serve as reactants in primitive metabolic pathways. They also propose that the iron sulfide membranes could provide a source of chemical energy, perhaps even chemiosmotic energy to drive early metabolism. This idea has merit and deserves further testing. In particular, it should be determined whether mineral membranes are able to act as true permeability barriers to the free diffusion of solutes. So far, permeability barriers have only been demonstrated in lipid bilayer membranes having a hydrophobic phase that has the capacity to maintain concentration gradients of polar and ionic solutes.

10
Self-Assembly Processes in Prebiotic Organic Mixtures

What physical properties are required if a molecule is to become incorporated into a stable bilayer? As discussed earlier, all bilayer-forming molecules are amphiphiles, with a hydrophilic “head” and a hydrophobic “tail” on the same molecule. If amphiphilic molecules were present in the mixture of organic compounds available on the early Earth, it is not difficult to imagine that their self-assembly into molecular aggregates was a common process.

Is this a plausible premise? In order to approach this question, we can assume that the mixture of organic compounds in carbonaceous meteorites such as the Murchison meteorite resembles components available on the early Earth through extraterrestrial infall. A series of organic acids represents the most abundant water-soluble fraction in carbonaceous meteorites [15, 67, 68]. Samples of the Murchison meteorite were extracted in an organic solvent commonly used to extract membrane lipids from biological sources [69, 70]. When this material was allowed to interact with aqueous phases, one class of compounds with acidic properties was clearly capable of forming membrane-bounded vesicles (Fig. 7).

Significantly, the photoproducts of interstellar ice simulations also include amphiphilic compounds having self-assembly properties [31]. Figure 8 shows micrographs of Murchison vesicles, as well as vesicles formed by products of interstellar ice simulations and known fatty acid–fatty alcohol mixtures. It is clear that the vesicle-forming behavior of all of these amphiphiles is

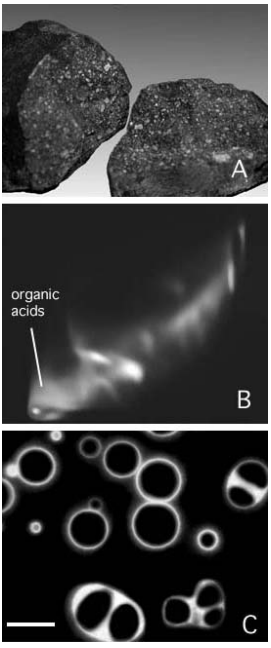


Fig. 7 Membranes can be formed by components of carbonaceous meteorites [69, 70]. **a** The Murchison meteorite contains approximately 2% organic carbon by weight. **b** Organic compounds can be extracted from the meteorite by a lipid solvent system (chloroform–methanol), then separated by two-dimensional chromatography. Polycyclic compounds in the mixture produce fluorescent spots. **c** The organic acid fraction from the TLC plate readily assembles into membranous vesicles when exposed to dilute aqueous solutions buffered at pH 8–9. The vesicles were photographed by their autofluorescence. *Scale bar* shows 20 μm

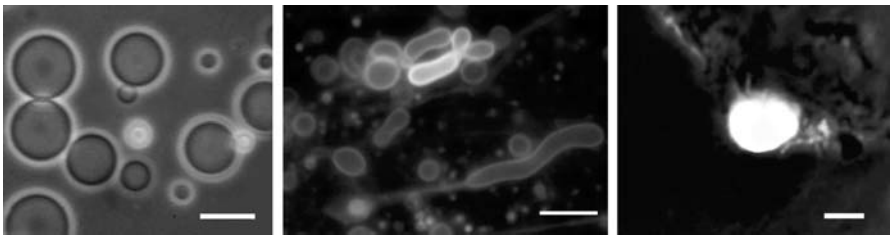


Fig. 8 Phase and fluorescence micrographs of membranous vesicular structures formed from a Murchison meteorite extract (*left*) compared to vesicles formed by a 20 mM decanoic acid–decanol mixture [72] (*center*) and a vesicular structure produced by the photoproduct of an interstellar-ice analog [31]. The vesicles produced by the photochemical ice analog product were allowed to capture pyranine, a fluorescent anionic dye, to demonstrate that a true membrane was present. *Scale bars* show 20, 10, and 5 μm, from *left* to *right*

similar. The organic compounds present in the meteoritic extract and those synthesized in the simulation of grain mantle photochemistry both contain amphiphilic compounds capable of self-assembly into membranous boundary structures. The vesicles produced from the interstellar simulations, like those of the meteoritic compounds, can also capture and maintain a gradient of ionic dye molecules.

From these results it is reasonable to conclude that a variety of simpler amphiphilic molecules were present on the early Earth that could participate in the formation of primitive membrane structures. The long chain acids and alcohols that contribute the amphiphilic property of contemporary membrane lipids are one possible component of prebiotic membrane structures. These compounds are present in carbonaceous meteorites [67, 68] and are synthesized under simulated geochemical conditions [11, 12]. Significantly, such simple amphiphiles can also form vesicles, as shown earlier [71, 72]. Stability of the vesicles is strongly dependent on chain length, concentration, amphiphile composition, temperature, and head group characteristics. For example, even a 9-carbon monocarboxylic acid—nonanoic acid—can form vesicles at concentrations of 85 mM and pH 7.0, which is the pK of the acid in bilayers [70]. Addition of small amounts of an alcohol (nonanol) further stabilizes the bilayers due to hydrogen bonding between the alcohol and acid head groups, so that vesicles can form at lower concentrations ($\sim$ 20 mM) at pH ranging from 6 to 11 (Fig. 4a). The vesicles provide a selective permeability barrier, as indicated by osmotic activity and ionic dye capture. As chain length increases, stability also increases and vesicles form at lower concentrations.

11
Environmental Constraints on the First Cell Membranes

Although self-assembly of amphiphilic molecules promotes the formation of complex molecular systems, the physical and chemical properties of an aqueous phase can significantly inhibit such processes, possibly constraining the environments in which cellular life first appeared. One such constraint is that temperature strongly influences the stability of vesicle membranes. It has been proposed that the last common ancestor, and even the first forms of life, were hyperthermophiles that developed in geothermal regions such as hydrothermal vents [60] or deep subterranean hot aquifers [61]. Such environments have the advantage of providing chemical energy in the form of redox potentials as well as abundant mineral surfaces to act as potential catalysts and adsorbants. However, because the intermolecular forces that stabilize self-assembled molecular systems are relatively weak, it is difficult to imagine how lipid bilayer membranes assembling from plausible prebiotic constituents would be stable under these conditions. All hyperthermophiles today have highly specialized lipid components, and it seems likely that these are the result of more recent adaptation than a molecular fossil of early life.

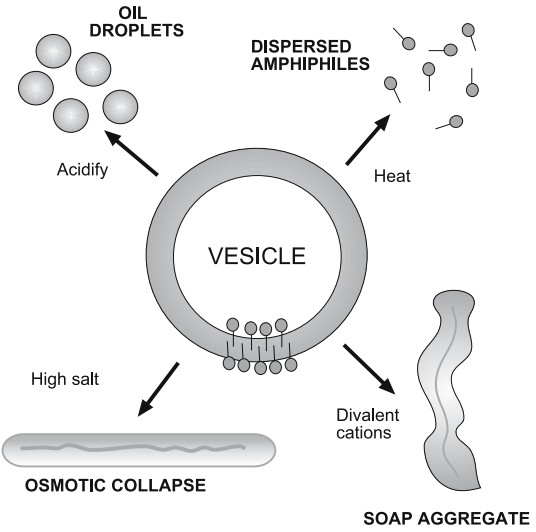


Fig. 9 Vesicles produced by single-chain amphiphiles such as fatty acids tend to be destabilized by certain environmental factors. If the fatty acid is protonated at low pH ranges, the membranes collapse into droplets. The vesicles also become increasingly unstable as temperature increases. In the presence of high salt concentrations, the vesicles undergo osmotic collapse and may also form nonmembranous aggregates if divalent cations react with the carboxylate head groups

A second concern is related to the ionic composition of a marine environment. The high salt concentration of the present ocean (near 0.5 M NaCl) has the potential to exert significant osmotic pressure on any closed membrane system (Fig. 9). All marine organisms today have evolved highly developed membrane transport systems that allow them to maintain osmotic equilibrium against substantial salt gradients across their membranes. Furthermore, the concentrations of divalent cations, in particular Mg^{2+} and Ca^{2+}, were likely to exceed 10 mM in the early oceans. In the absence of oxygen, Fe^{2+} would also be present at similar concentrations. All such divalent cations have a strong tendency to bind to the anionic head groups of amphiphilic molecules, strongly inhibiting their ability to form stable membranes [73].

These considerations suggest that, from the perspective of membrane biophysics, the most plausible planetary environment for the origin of life would be at moderate temperature ranges (< 60 °C), and the ionic content would correspond to low ionic strength and pH values near neutrality (pH 5–8) with divalent cations at submillimolar concentrations. This suggestion is in marked contrast to the view that life most likely began in a marine environment, perhaps even the extreme environment of a hydrothermal vent. A marine site for life's beginning seems plausible because freshwater would be rare on the early Earth. Even with today's extensive continental crust, freshwater only represents ~ 1% of the contemporary Earth's reservoir of liquid water. Another concern about a freshwater origin of life is that the lifetime of freshwater bodies tends to be geologically short-lived. On the other hand, if seawater, with its high content of sodium chloride and divalent ions, markedly inhibits self-assembly processes and reactions that are essential to the emergence of cellular life, we may need to reconsider the assumption that life inevitably began in a marine environment. A more plausible site for the origin of cellular life may be a low-ionic-strength lacustrine environment such as a pond or lake. After the first form of cellular life was able to establish itself in a relatively benign environment, it would rapidly begin to adapt through Darwinian selection to more rigorous environments, including the extreme temperatures, salt concentrations, and pH ranges that we associate with the limits of life on the Earth.

12
Model Systems of Primitive Cells

A central event in the origin of life was the self-assembly of a molecular system in which catalytic polymers could interact with a second class of polymers having the capacity to store information in a sequence of monomers. That sequence in turn would in some manner determine the sequence of monomers in the catalyst, so that the resulting catalytic-information cycle

was able to undergo directed growth. In contemporary cells, the cycle is represented by protein catalysts (enzymes) and nucleic acids that store genetic information and have the potential to transmit that information to a second molecule by replication or transcription. However, in a protocell, both catalytic and information-containing sites could be present in the same molecule, as suggested by recent studies of RNA ribozymes [74]. Several approaches to artificial cells have been proposed to test various scenarios for the origin of cellular life [75–78]. An ideal model cell would incorporate an encapsulated polymerase activity together with a template of some sort, so that sequence information in the template could be transcribed to a second molecule. The membrane must be sufficiently permeable to allow the polymerase to have access to externally added substrates. Furthermore, the membrane itself should be able to grow in order to accommodate the growth of the encapsulated polymers. Finally, in an ideal cell model, the polymerase itself would be reproduced from information in the template, so that the entire system would be able to grow and evolve.

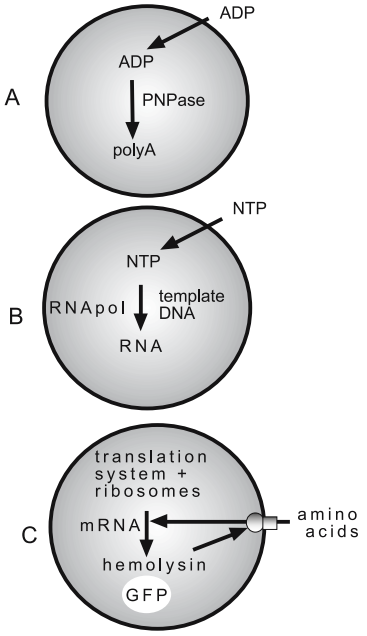


Fig. 10 Model protocell systems. **a** An encapsulated polymerase (polynucleotide phosphorylase) can synthesize RNA from nucleoside diphosphates such as ADP [79, 80]. **b** RNA can be synthesized by a template-dependent T7 RNA polymerase [83]. **c** Proteins such as green fluorescent protein (GFP) can be synthesized by an encapsulated translation system [84]. If mRNA coding for hemolysin is also present, the hemolysin forms a pore in the lipid bilayer. Amino acids then permeate the bilayer, and protein synthesis can continue for several days [85]

To demonstrate polymerase activity in a model cell, Chakrabarti et al. [79] encapsulated polynucleotide phosphorylase in vesicles composed of dimyristoylphosphatidylcholine (DMPC). This enzyme can produce RNA from nucleoside diphosphates such as adenosine diphosphate (ADP) and does not require a template, so it has proven useful for initial studies of encapsulated polymerase activity (Fig. 10a). Furthermore, DMPC liposomes are sufficiently permeable so that 5–10 ADP molecules per second enter each vesicle. Under these conditions, measurable amounts of RNA in the form of polyadenylic acid were synthesized and accumulated in the vesicles after several days' incubation. The enzyme-catalyzed reaction could be carried out in the presence of a protease external to the membrane, demonstrating that the vesicle membrane protected the encapsulated enzyme from hydrolytic degradation. Similar behavior has been observed with monocarboxylic acid vesicles [80], and it follows that complex phospholipids are not required for an encapsulated polymerase system to function.

In other work, the Q-beta replicase [81] and the components of the polymerase chain reaction (PCR) [82] have also been encapsulated, together with templates and substrates in the form of nucleoside triphosphates (NTPs), and are functional in liposomes. Both of these enzyme systems use templates, so it is clear that template-dependent polymer synthesis can occur in an encapsulated environment. The phospholipids used in these studies were relatively impermeable, so substrates were necessarily encapsulated along with enzyme and template. This limited the amount of nucleic acid replication that could occur to a few molecules per vesicle. However, the permeability barrier can in principle be overcome by introducing transient defects in the membranes of lipid vesicles. For instance, a template-directed reaction can be encapsulated in DMPC liposomes in which externally added substrates were used to supply the enzyme [83]. In this study, T-7 RNA polymerase and a circular 4000 bp plasmid template were encapsulated, and substrates were provided by addition of the NTPs (Fig. 10b). The system was subjected to temperature cycles of 23 °C and 37 °C in a PCR apparatus. DMPC membranes are relatively permeable at the phase transition temperature of 23 °C, permitting substrate ribonucleotides to enter the vesicles, while at 37 °C the membranes become much less permeable but the polymerase is activated. RNA synthesis was monitored by incorporation of radiolabeled UTP, and transcription was confirmed by reverse PCR. Figure 11 shows a micrograph of the resulting structures containing RNA synthesized within the vesicle volume.

Most recently, functioning translation systems that included ribosomes have been encapsulated in lipid vesicles. The first attempt to assemble a translation system in a lipid vesicle system was made by Oberholzer et al. [84]. However, only very small amounts of peptides were synthesized, largely because the lipid bilayer was impermeable to amino acids, so that ribosomal translation was limited to the small number of amino acids encapsulated within the vesicles. Yu et al. [85] and Nomura et al. [86] improved the yield

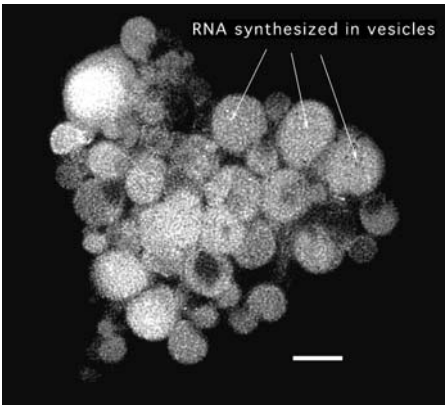


Fig. 11 Lipid vesicles with encapsulated T7 RNA polymerase and DNA template. A mixture of four nucleoside triphosphates was added, and these diffused into the vesicles and were used by the polymerase to synthesize RNA with DNA as a template. The RNA was stained with ethidium bromide and appears as fluorescent material within the vesicles. Note that some of the vesicles do not contain fluorescent RNA, presumably because they lacked sufficient enzyme or template. *Scale bar* shows 20 μm

substantially by using larger vesicles, demonstrating that green fluorescent protein can be synthesized by an encapsulated translation system. Noireaux and Libchaber [87] took this approach one step further by incorporating two genes into a similar encapsulated translation system, one for alpha hemolysin, a pore-forming protein, and a second for GFP. The investigators reasoned that if the system was in fact capable of synthesizing proteins, the newly translated hemolysin would insert into the membrane and produce a pore. This would allow externally added solutes (i.e., amino acids) to permeate the lipid bilayer barrier and supply the substrates required for protein synthesis. The production of GFP would then indicate that synthesis was proceeding at appreciable rates. This worked very well, and GFP could be visualized accumulating in the vesicular volume for up to 4 d.

The next obvious step is to incorporate both a gene transcription system and protein synthesis in lipid vesicles. This was reported in 2004 by Ishikawa et al. [87], who managed to assemble a two-stage genetic network in liposomes in which the gene for an RNA polymerase was expressed first and the polymerase then used to produce mRNA required for GFP synthesis.

The final challenge in modeling such systems will be to encapsulate an evolving ribozyme system [74, 86, 87] within vesicles formed from amphiphilic mixtures that are optimized for stability and permeability. It seems likely that one such mixture will have a set of properties that permit it to encapsulate a catalytic polymerase system and template, with sufficient permeability to allow substrate access to the enzyme at reasonable rates. Replication and ribozyme evolution would then occur in immensely large numbers

of microscopic volumes represented by the liposome interiors, rather than in the macroscopic volume of a test tube. Under these conditions, the rare ribozyme that happens to undergo a favorable mutation would be readily selected, whereas in a test tube it is lost among trillions of other similar molecules.

13 Summary

This chapter provides a perspective on the most primitive forms of cellular life. In the early Earth environment, there must have been a variety of amphiphilic hydrocarbon derivatives that could self-assemble into bilayer boundary structures and encapsulate polymers that were being synthesized by a separate process. The vesicle membranes would have been sufficiently permeable to allow passage of smaller ionic substrates required for metabolism and biosynthesis, yet maintain larger molecules within. Encapsulated catalysts and information-bearing molecules would thus have had access to nutrients required for growth. Furthermore, specific groupings of macromolecules would be maintained, rather than drifting apart. This would allow true Darwinian-type selection of such groupings to occur, a process that could not take place in mixtures of molecules free in solution. A small number of the encapsulated molecular systems were likely to have the specific set of properties that allowed them to capture free energy and nutrients from their environment and undergo growth by polymerization. At some point, the growth would become catalyzed by the encapsulated polymers and then begin to be directed by a primitive genetic process. Such structures would be on the evolutionary path to the first forms of cellular life.

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