

Review

The First Cell Membranes

DAVID DEAMER,¹ JASON P. DWORKIN,^{2,3} SCOTT A. SANDFORD,²
MAX P. BERNSTEIN,^{2,3} and LOUIS J. ALLAMANDOLA²

ABSTRACT

Organic compounds are synthesized in the interstellar medium and can be delivered to planetary surfaces such as the early Earth, where they mix with endogenous species. Some of these compounds are amphiphilic, having polar and nonpolar groups on the same molecule. Amphiphilic compounds spontaneously self-assemble into more complex structures such as bimolecular layers, which in turn form closed membranous vesicles. The first forms of cellular life required self-assembled membranes that were likely to have been produced from amphiphilic compounds on the prebiotic Earth. Laboratory simulations show that such vesicles readily encapsulate functional macromolecules, including nucleic acids and polymerases. The goal of future investigations will be to fabricate artificial cells as models of the origin of life. Key Words: Membranes—Amphiphiles—Self-assembly—Prebiotic. *Astrobiology* 2, 371–381.

INTRODUCTION

MEMBRANOUS BOUNDARY STRUCTURES define all life today, and a source of membrane-bounded microenvironments on the early Earth was essential for the rise of cellular life. Recent evidence suggests that membranes can self-assemble from organic mixtures available on any planet having liquid water and a source of organics (Dworkin *et al.*, 2001). It now appears likely that extraterrestrial material falling on the primordial Earth was a source not only of the elements from which prebiotic molecules were formed, but also of specific organic compounds that harbored the first cellular forms of life.

Here we review the origin and potential sig-

nificance of complex organic molecules in the prebiotic environment. We first present evidence that abiotic production of biologically relevant substances is widespread in the interstellar medium (ISM), and that some of these molecules reach planetary surfaces intact, where they mix with compounds synthesized on the planet. After describing possible sources and varieties of such compounds, we will discuss self-assembly processes that have the potential to produce increasingly complex molecular systems under conditions prevalent on the early Earth. Such self-assembly processes may have ushered in the emergence of a membrane-bound self-reproducing molecular system and the origin of cellular life.

¹Department of Chemistry and Biochemistry, University of California at Santa Cruz, Santa Cruz, California.

²NASA-Ames Research Center, Moffett Field, California.

³Center for the Study of Life in the Universe, SETI Institute, Mountain View, California.

SOURCES OF PREBIOTIC ORGANIC COMPOUNDS

Following the Urey–Miller synthesis of amino acids by spark discharge in a mixture of reduced gases (Miller, 1953), formation of prebiotic organic compounds on the early Earth became central to postulated scenarios for the origin of life (Wills and Bada, 2000). Numerous endogenous synthetic pathways have been investigated, and a few examples include reactions in the atmosphere (Pinto *et al.*, 1980), in aqueous phases and on mineral surfaces (Wächtershäuser, 1988; Cody *et al.*, 2000), and in mineral matrices (Freund and Ho, 2002). However, the yields are sensitive to environmental conditions that are not well constrained (Chang, 1988; Kasting and Brown, 1998), so that their relative contribution to the prebiotic organic inventory is uncertain. For the most part, studies of endogenous synthesis have focused on the production of water-soluble polar compounds such as amino acids, simple sugars, and nucleic acid bases. Amphiphilic products, defined as membrane-forming molecules containing both polar and nonpolar groups on the same structure, have received less attention. Such amphiphilic molecules are thought to be products of Fischer–Tropsch reactions catalyzed by metal or mineral surfaces (McCollom *et al.*, 1999; Rushdi and Simoneit, 2001) in the presolar nebula or on the planetary surface.

Exogenous delivery of intact extraterrestrial molecules is a second source of prebiotic organic material to the primordial Earth. These organic molecules are formed as a by-product of the cycle of stellar birth and death. The most important biogenic elements (C, N, O, S, and P) form in the interiors of stars, then are ejected into the surrounding ISM at the end of the star's lifetime during red giant, nova, and supernova phases. Following ejection, carbon-containing material disperses into the surrounding diffuse ISM, where it is modified by a variety of physical and chemical processes including UV irradiation, cosmic ray bombardment, gas-phase chemistry, accretion and reaction upon grain surfaces, and destruction by interstellar shock waves such as those generated by supernovae. Eventually, much of this material becomes concentrated into dense molecular clouds from which new stars and planetary systems are formed (Sandford, 1996; Ehrenfreund and Charnley, 2000).

Dense molecular clouds attenuate the harsh in-

terstellar radiation field, permitting the synthesis and survival of more complex species in the gas phase than is possible in the diffuse ISM. At the low temperatures in these dark molecular clouds (10–50K), 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. Comparisons of infrared spectra of low-temperature laboratory ices with absorption spectra of molecular clouds indicate that interstellar ices are mainly composed of H₂O mixed with CO, CO₂, CH₃OH, NH₃, and other components, with the latter ingredients generally making up 5–15% 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), the attenuated diffuse ISM UV field, and UV photons from stars forming within the cloud.

Laboratory experiments have shown that radiation processing of presolar ices leads to more complex molecular species (Bernstein *et al.*, 1995; Gerakines *et al.*, 2000; Ehrenfreund *et al.*, 2001). 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 relevant to the origin of life, including amino acids (Bernstein *et al.*, 2001, 2002; Muñoz Caro *et al.*, 2002) and amphiphilic material (Dworkin *et al.*, 2001). The fact that quinones are present among the products raises the intriguing possibility that exogenous materials could have delivered not only molecules with properties related to the molecular structure of cells, but also certain coenzyme-like compounds that could participate in electron transport functions required for the generation of reducing power and ion gradients.

The production of relevant organic molecules in the ISM is of little consequence to the origin of life unless these molecules can be delivered intact to habitable planetary surfaces. 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 (Pierazzo and Chyba, 1999). More directly, there is am-

ple physical evidence that some organic species do, in fact, survive planetary accretion, with the best evidence coming from deuterium isotopic measurements of meteorites and 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 (Krishnamurthy *et al.*, 1992). 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 (Sandford *et al.*, 2001).

Meteorites and IDPs deliver organic materials to the modern Earth at a rate of $\sim 10^7$ kg/year (Love and Brownlee, 1993). During the late bombardment period, which lasted until ~ 4 billion years ago, the amount of extraterrestrial organic material brought to the prebiotic Earth was likely to have been orders of magnitude greater (Chyba and Sagan, 1992). Thus, the early Earth must have been seeded with organic matter created in the ISM, protosolar nebula, and asteroidal/cometary parent bodies (Fig. 1).

From these considerations, it seems an inescapable conclusion that both exogenous delivery and endogenous synthetic pathways provided organic material to the prebiotic environment important to the origin of life. 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. Critical among these are amphiphilic compounds that have the capacity to self-assemble into more complex structures. The rest of this review discusses the properties of such compounds and how they may have formed the first cell membranes.

SELF-ASSEMBLY AND LIFE'S ORIGINS

Life has been defined as a chemical system capable of Darwinian evolution (Joyce and Orgel, 1998), and by this definition the propagation of information is a key issue. A fundamental goal of research into the origin of life has been to understand the nature of the first macromolecule capable of containing information in the sequence of its monomers (Joyce and Orgel, 1993). Because of the central position of genes and catalysts in

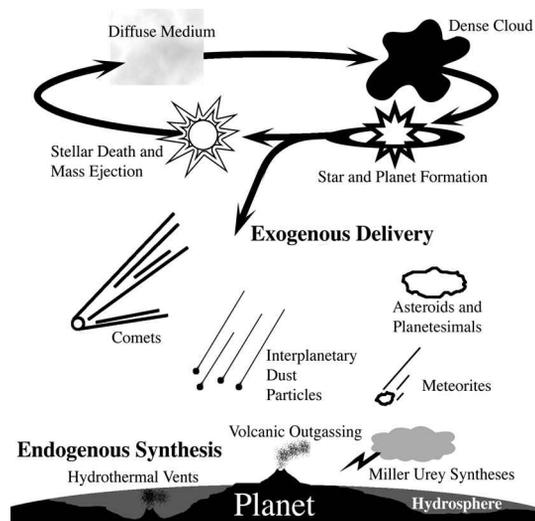


FIG. 1. A cycle of stellar birth and death leads to the synthesis and evolution of organic compounds that can be delivered intact to and mixed with those produced on planetary surfaces.

contemporary life, a common view among workers in the field has been that life began with the formation of a polymer having both catalytic capacity and the ability to contain and propagate information. The concept of an RNA world, in which life was mediated by RNA taking on the roles held by proteins and DNA in modern biology, is compelling (Joyce and Orgel, 1993; Schwartz, 1998). However, plausible prebiotic synthesis of activated monomers and their subsequent polymerization remain elusive, and it is now clear that an RNA world (or even its molecular precursor, pre-RNA) would be difficult to achieve directly from simple organic molecules dissolved in a global ocean (Joyce, 1991). Even if it were possible to generate chemically activated nucleotides capable of polymerizing into RNA in solution, in the absence of some concentrating mechanism these would be greatly diluted, and no further reactions could occur. To address this problem, it was proposed that concentration might occur by processes such as evaporation in lagoons (Robertson and Miller, 1995), eutectic freezing (Stribling and Miller, 1991; Kanavarioti *et al.*, 2001), or adsorption to mineral surfaces (Wächtershäuser, 1988; Ferris *et al.*, 1996; Sowerby *et al.*, 2002). These physical mechanisms may have been important for the synthesis and accumulation of complex materials on Earth, but their inherent inefficiencies would seem to be inconsistent with moving beyond the initial stages of

generating monomers and perhaps random polymers.

Rather than focusing on a single molecular species that incorporates the primary features of life, an alternative scenario is that the origin of life involved the self-assembly of molecular systems within a variety of cell-sized environments (Koch, 1985; Morowitz, 1992; Deamer, 1997; Segré *et al.*, 2001). Self-assembly in liquid water occurs when small amphiphilic molecules spontaneously associate by hydrophobic interactions into more complex structures with defined compositions and organization. Examples include the assembly of amphiphilic molecules into micelles, monolayers, and bilayers in the form of vesicles (Fig. 2).

Solutions inside such structures are highly concentrated relative to the bulk phase, thereby overcoming the dilution that occurs with water-soluble organic species. Membrane-bounded structures are capable of maintaining specific groups of macromolecules within, facilitating their interaction and promoting a form of speciation that is lacking in bulk-phase environments. Membranes also have the potential to maintain concentration gradients of ions, thus providing a source of free energy that can drive transport processes across the membrane boundary. Finally, if certain components of the prebiotic organic inventory happened to be nonpolar pigments, they would partition into the hydrophobic phase of a membrane and have the potential to capture visible light energy and protect other components from damaging UV light. This emergent set of biophysical properties can only arise

from amphiphilic molecules that have the ability to self-assemble into more complex vesicular structures.

The discovery that biologically relevant compounds such as amino acids are readily synthesized under simulated early Solar System conditions inspired a major effort to determine whether other biologically significant compounds are present in known samples of extraterrestrial material, particularly carbonaceous meteorites. Such meteorites contain up to several percent of their mass as organic carbon, primarily as an organic polymer composed of polycyclic aromatic hydrocarbon (PAH) but also containing a variety of water-soluble compounds including amino acids (Cronin *et al.*, 1988). In addition, and more important for the current discussion, material extracted from the Murchison meteorite by organic solvents contains amphiphiles that form membranous vesicles in aqueous solutions (Fig. 3A) (Deamer, 1985; Deamer and Pashley, 1989). The vesicles derived from Murchison extracts are fluorescent, suggesting that both amphiphiles and PAH derivatives are present in the mixture. It is likely that the PAH derivatives contribute to the stability of the membranes, just as the polycyclic aliphatic molecule cholesterol does in biological membranes. Significantly, the membranes have the ability to act as a permeability barrier and thereby capture ionic solutes such as fluorescent dye molecules (Fig. 3A). This property is a central function of all biological membranes, and would be essential for any self-assembled membrane that formed the boundary of primitive cells.

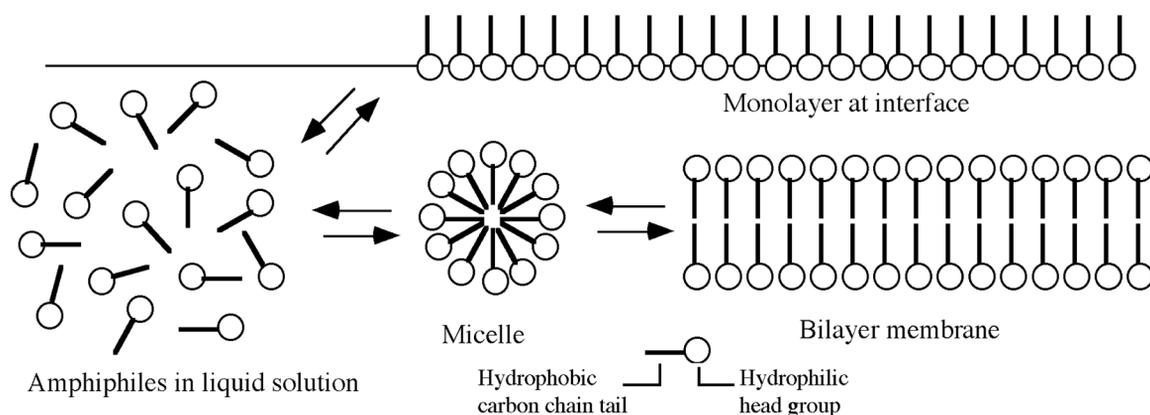


FIG. 2. Self-assembled structures of amphiphiles. Amphiphilic molecules having carbon chain lengths greater than six carbons form micelles as concentrations increase above a critical value. At chain lengths of eight carbons and higher, bilayers begin to appear in the form of membranous vesicles, which become the dominant structure as concentrations increase further.

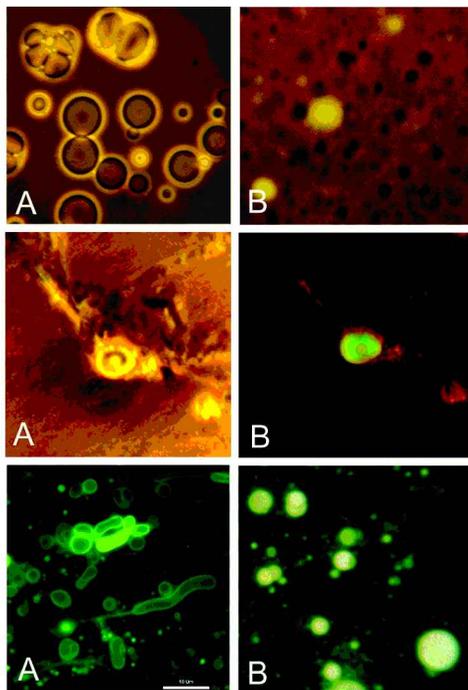


FIG. 3. Phase (A) and fluorescence (B) micrographs of membranous vesicular structures formed from a Murchison meteorite extract (Deamer, 1985; Deamer and Pashley, 1989, upper panel) compared with a vesicular structure produced in the extract from an interstellar ice analog (Dworkin *et al.*, 2001, center panel). Both the meteoritic vesicles and those synthesized photochemically from compounds in the ice analog have the ability to capture pyranine, a fluorescent anionic dye. **Lower panel:** Vesicles formed by a 20 mM decanoic acid/decanol mixture (1:1) at pH 8.0. Such vesicles readily encapsulate macromolecules as large as ~600-bp double-stranded DNA, shown on the right. The DNA has been stained with acridine orange.

As noted previously, interstellar ice simulations show that amphiphilic compounds can be produced in the ISM (Dworkin *et al.*, 2001). Figure 3 (center panel) shows a photomicrograph of the fluorescent self-assembled structures that are produced by amphiphilic compounds made by the irradiation of the interstellar/precometary ice $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{NH}_3:\text{CO}$ (100:50:1:1) at ~15K. The fluorescence arises from a variety of complex organic molecules that are produced along with the amphiphilic compounds within the ice by UV irradiation. As shown in Fig. 3, the vesicle-forming behavior of these amphiphiles is similar to that displayed by organic components of the Murchison meteorite (Deamer and Pashley, 1989). The organic compounds present in the meteoritic extract and those synthesized in the simulation of grain mantle photochemistry 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 (Dworkin *et al.*, 2001). The properties described above for both meteoritic and synthetic mixtures of amphiphilic compounds clearly demonstrate the potential significance of self-assembly for primitive membrane structures related to the origin of cellular life. However, in each case, only microscopic quantities of material are available, and for this reason model systems are being developed as simulations to study prebiotic self-assembly. Such systems were first investigated as microscopic gel structures called coacervates (Oparin *et al.*, 1976) and proteinoid microspheres (Fox, 1973). However, neither coacervates nor proteinoid microspheres have a true boundary membrane that can act as a selective permeability barrier. A more useful model is based on liposomes, defined as microscopic vesicles composed of one or more lipid bilayers that encapsulate a volume (Bangham, 1981). Liposomes are typically prepared from biological or synthetic phospholipids (Fig. 4), sometimes with the admixture of cholesterol as a stabilizing agent.

CONSTRAINTS ON THE FIRST CELL MEMBRANES

Although phospholipids are universal components of cell membranes today, it seems improbable that such complex molecules were available on the prebiotic Earth. It is more likely that simpler amphiphilic molecules were present that could participate in the formation of primitive membrane structures. It has been proposed (Ourison and Nakatani, 1994) that amphiphiles based on isoprene derivatives were components of primitive cell membranes. Although this is consistent with molecular fossil evidence from evolutionarily deep branching Archaean microbial populations, no prebiotic synthetic pathway to such compounds has yet been demonstrated.

The long-chain acids and alcohols that contribute the amphiphilic property of contemporary membrane lipids are another possible component of prebiotic membrane structures. These compounds are present in carbonaceous meteorites (Lawless and Yuen, 1979; Naraoka *et al.*, 1999) and have been synthesized under simulated geo-

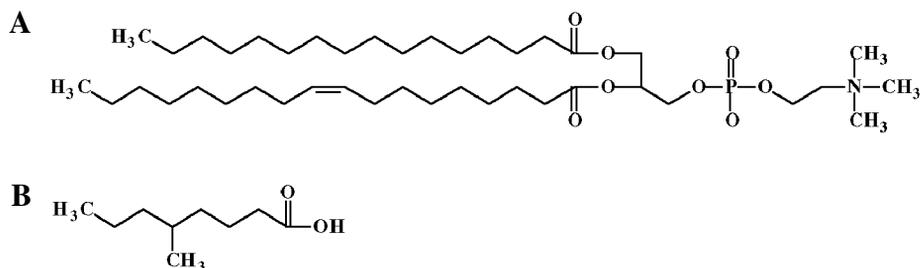


FIG. 4. A modern membrane phospholipid (1-palmitoyl-2-oleoyl-phosphatidylcholine) (A) compared with a typical amphiphilic compound present in carbonaceous meteorites (5-methylnonanoic acid) (B). Phospholipids of biomembranes usually have one or more *cis*-unsaturated bonds in their hydrocarbon chains to assure that bilayers remain fluid at physiological temperatures. The monocarboxylic acids of meteorites typically have branched chains.

chemical conditions that are models of hydrothermal systems at high pressures and temperatures. (McCullom *et al.*, 1999; Rushdi and Simoneit, 2001). Significantly, such simple amphiphiles can also form vesicles, as shown in Fig. 3 (lower panel) (Hargreaves and Deamer, 1978; Apel *et al.*, 2002). Stability of the vesicles is strongly dependent on chain length, concentration, amphiphile composition, temperature, and head group characteristics. For example, even a nine-carbon monocarboxylic acid—nonanoic acid—can form stable vesicles at concentrations of 85 mM and pH 7.0, which is the pK of the acid in bilayers (Apel *et al.*, 2002). At pH 6 and below, the acid group is protonated, and the vesicles become unstable as the nonanoic acid increasingly forms droplets. At pH ranges of 8 and above, vesicles are lost, and clear solutions of micelles are present. However, addition of small amounts of an alcohol (nonanol) stabilizes the bilayers owing to hydrogen bonding between the alcohol and acid head groups, and vesicles can form at lower concentrations (~20 mM) at pH ranging from 6 to 11. 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.

Because the amphiphilic components of the Murchison meteorite organic extract and the organic products of ice photolysis are highly complex mixtures and available only in limited quantities, the exact composition of the membrane-forming amphiphiles has not yet been established in detail. However, it is clear that monocarboxylic acids and polycyclic aromatic compounds are among the major membrane-forming components in Murchison extracts. We

suggest that the first boundary structures on the early Earth assembled from such mixtures, and that they are worth further investigation as models of the first membranes. It is significant that at least one biological membrane is composed entirely of single-chain amphiphiles that consist of a mix of chlorosulfolipids, fatty acids, and cholesterol (Chen *et al.*, 1976), indicating that single-chain amphiphiles can serve as boundary structures in the membranes of contemporary cells as well.

An important consideration here is that a boundary membrane potentially isolates a primitive catalytic replicating system from the nutrients required for growth. For instance, the permeability of phospholipid membranes to ionic nutrients such as amino acids, nucleotides, and phosphate is in the range of 10^{-11} – 10^{-12} cm/s (Chakrabarti and Deamer, 1994), a value so low that only a few solute ions can cross the lipid bilayer of a given vesicle per minute. In contrast, a bacterial cell can take up millions of nutrient solute molecules per second during active growth, using specialized membrane proteins such as permeases and pumps to facilitate nutrient transport across the lipid bilayer barrier.

In the absence of such highly evolved transport proteins, how might an early form of cellular life gain access to nutrient solutes? One possibility is that primitive membranes composed of simple amphiphiles were significantly more permeable to ionic solutes. For example, if the chain lengths of phospholipids composing a lipid bilayer are reduced from the 18 carbons of modern biological membranes to 14 carbons, thereby thinning the membrane, the permeability to ionic solutes increases by three orders of magnitude (Paula *et al.*, 1996). This is sufficient to allow molecules as

large as ATP to cross the permeability barrier at a useful rate, while still maintaining macromolecules in the encapsulated environment (Monnard and Deamer, 2001). Thus, it seems reasonable to suggest that the earliest biologically important membranes may have been composed of mixtures of amphiphiles with relatively short chain lengths. Such membranes could capture and concentrate macromolecules, yet still provide access to ionic nutrient solutes in the external aqueous phase. At some point early in evolution, a primitive transport system would have to evolve, perhaps in the form of a polymeric compound that can penetrate the bilayer structure and provide a channel. It is interesting to note that selected RNA species have been demonstrated to interact with lipid bilayers and produce ion-conducting channels (Vlassov *et al.*, 2001).

PRIMITIVE CELLS IN THE ARCHAEOAN ENVIRONMENT

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 (Deamer and Barchfeld, 1982) or by freeze–thaw cycles (Pick, 1981). 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 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. 3, lower panel). Several enzymes have also been encapsulated using similar procedures (Nasseau *et al.*, 2001).

Although self-assembly of amphiphilic molecules promotes the formation of complex molecular systems, the physical and chemical properties of aqueous environments 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 mem-

branes. 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 (Baross and Hoffman, 1985) or deep subterranean hot aquifers (Pace, 1991). 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.

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) can exert significant osmotic pressure on any closed membrane system. All marine organisms today have evolved active transport systems that allow them to maintain osmotic equilibrium against substantial salt gradients across their membranes. In addition to sodium chloride, the concentrations of divalent cations such as 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 (Monnard *et al.*, 2002).

These considerations lead us to suggest that, from the perspective of membrane biophysics, the most plausible planetary environment for the origin of life would be at moderate temperature ranges ($<60^{\circ}C$), 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 a marked departure from the widely held 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 fresh water would be rare on the early Earth. Even with today's extensive continental crust, fresh water only represents $\sim 1\%$ of contemporary Earth's reservoir of liquid water. Another concern about a freshwa-

ter origin of life is that the lifetime of freshwater bodies tends to be short on a geological time scale. However, it should be noted that the residence time of organic compounds in the ancient ocean would also be limited by destructive cycling through hydrothermal vents (Miller and Lazcano, 1995).

Further experimental work may establish that certain amphiphilic mixtures can in fact form stable self-assembled molecular systems in high-temperature aqueous phases containing divalent cation concentrations similar to seawater. However, until we have such experimental systems in hand, it seems reasonable to propose that a more plausible site for the origin of cellular life would be a low ionic strength lacustrine environment such as a pond or lake. After the first forms of cellular life were established in relatively benign environments, they would have rapidly begun 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 today.

MODEL SYSTEMS OF PRIMITIVE CELLS

Several concepts for models of cells have been proposed to test various scenarios for the origin of cellular life (Cavalier-Smith, 1987; Luisi, 1998; Szostak *et al.*, 2001; Pohorille and Deamer, 2002). A typical model cell incorporates an encapsulated polymerase activity together with a template of some sort, so that sequence information in the template can 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 is able to grow and evolve.

Substantial progress has been made in laboratory investigations of such systems. For instance, Luisi and co-workers (Bachman *et al.*, 1992) have shown that vesicles composed of oleic acid can grow and "reproduce" as oleoyl anhydride spontaneously hydrolyzes in the reaction mixture, thereby adding additional amphiphilic components (oleic acid) to the vesicle membranes. To

demonstrate polymerase activity in a model cell, Chakrabarti *et al.* (1994) encapsulated polynucleotide phosphorylase in vesicles composed of dimyristoylphosphatidylcholine (DMPC). This enzyme can produce RNA from nucleoside diphosphates such as ADP and does not require a template, so it has proven useful for initial studies of encapsulated polymerase activity. Furthermore, DMPC liposomes are sufficiently permeable so that five to 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 of 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 (Walde *et al.*, 1994), so phospholipids are not required for an encapsulated polymerase system.

In other work, the Q-beta replicase (Oberholzer *et al.*, 1995a) and the components of the polymerase chain reaction (Oberholzer *et al.*, 1995b) have also been encapsulated, together with templates and substrates in the form of nucleoside triphosphates, 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 that 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. More recently, a template-directed reaction was established in DMPC liposomes in which external substrate was used to supply the enzyme (Monnard and Deamer, 2002). In this study, T-7 RNA polymerase and a circular 4,000-bp plasmid template were encapsulated, and substrates were provided by addition of the ribonucleotides ATP, GTP, CTP, and UTP. RNA synthesis was monitored by incorporation of radiolabeled UTP, and transcription was confirmed by reverse polymerase chain reaction.

An important next step in modeling such systems will be to encapsulate an evolving ribozyme system (Beaudry and Joyce, 1992; Wilson and Szostak, 1994; Johnston *et al.*, 2001) within vesicles formed from amphiphilic mix-

tures that are optimized for stability and permeability. It seems likely that one such mixture will have an optimal 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 an immensely large number 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.

In summary, we have proposed here that exogenous delivery and endogenous synthesis are potentially important sources of prebiotic and biogenic molecules on the early Earth. Both processes can provide amphiphilic molecules capable of self-assembly into membrane structures. Under conditions prevalent on the early Earth, assembly of membranous vesicles, followed by incorporation and development of increasingly complex polymeric systems, would enable the emergence of a membrane-bounded self-reproducing molecular system. Taking the physical properties of such systems into account, the ideal environment for the production and stabilization of early cellular life would include moderate temperature ranges, pH in the neutral range, and low ionic strength. This approach to developing laboratory models of primitive cells should help us better understand the evolutionary pathway that led to the first forms of cellular life.

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ABBREVIATIONS

DMPC, dimyristoylphosphatidylcholine; IDP, interplanetary dust particle; ISM, interstellar medium; PAH, polycyclic aromatic hydrocarbon.

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Address reprint requests to:

Dr. David Deamer

Department of Chemistry and Biochemistry

University of California at Santa Cruz

Santa Cruz, CA 95064

E-mail: deamer@hydrogen.ucsc.edu