Cold Spring Harbor Symposia on Quantitative Biology

Reconstructing the Emergence of Cellular Life through the Synthesis of Model Protocells

S.S. Mansy and J.W. Szostak

Cold Spring Harb Symp Quant Biol published online September 4, 2009
Access the most recent version at doi:10.1101/sqb.2009.74.014

Reconstructing the Emergence of Cellular Life through the Synthesis of Model Protocells

S.S. MANSY[1] AND J.W. SZOSTAK[2]

[1]*Armenise-Harvard Laboratory of Synthetic and Reconstructive Biology, Centre for Integrative Biology, University of Trento, 38100 Mattarello (Trento), Italy;* [2]*Howard Hughes Medical Institute, and Department of Molecular Biology and Center for Computational and Integrative Biology, Massachusetts General Hospital, Simches Research Center, Boston, Massachusetts 02115*

Correspondence: szostak@molbio.mgh.harvard.edu

The complexity of modern biological life has long made it difficult to understand how life could emerge spontaneously from the chemistry of the early earth. The key to resolving this mystery lies in the simplicity of the earliest living cells, together with the ability of the appropriate molecular building blocks to spontaneously self-assemble into larger structures. In our view, the two key components of a primitive cell are not only self-assembling, but also self-replicating, structures: the nucleic acid genome and the cell membrane. Here, we summarize recent experimental progress toward the synthesis of efficient self-replicating nucleic acid and membrane vesicle systems and discuss some of the issues that arise during efforts to integrate these two subsystems into a coherent whole. We have shown that spontaneous nucleic-acid-copying chemistry can take place within membrane vesicles, using externally supplied activated nucleotides as substrates. Thus, membranes need not be a barrier to the uptake of environmentally supplied nutrients. We examine some of the remaining obstacles that must be overcome to enable the synthesis of a complete self-replicating protocell, and we discuss the implications of these experiments for our understanding of the emergence of Darwinian evolution and the origin and early evolution of cellular life.

The differences between chemical evolution and Darwinian evolution are clear-cut at the extremes, but they are less distinct at the interface where complex chemistry transforms into simple biology. A careful consideration of the transition from chemistry to biology reveals a series of stages, in which the inheritance of variation and the natural selection of advantageous phenotypes become progressively more powerful and open-ended. For example, the sequences of the first polynucleotides would have been biased by simple chemical considerations such as the concentration and intrinsic reactivities of the available monomers, and different sequences would have been degraded at rates dependent on chemical factors such as the intrinsic lability of different linkages, as well as the tendency of certain sequences to fold into more stable secondary structures. The sequence distribution of the population would thus change with time, due to differences in the rate of synthesis and degradation. At this level, we can see the emergence of genetically determined phenotypes, including differential survival, but the transmission of variation from generation to generation is still missing. This critical property comes into play as soon as some mechanism for template-directed replication arises. Because some sequences would undoubtedly be better templates than others, there would certainly be natural selection for sequences that were replicated more efficiently. This could include subtle competing effects such as selection for secondary structures that slow down reannealing with complementary strands while not being too difficult to replicate. Nevertheless, it seems unlikely that template copying in solution could lead to more complex outcomes, such as the emergence of catalysts of replication or metabolic reactions. To attain this highest and most open-ended level of natural selection, we have argued previously that spatial compartmentalization is required (Szostak et al. 2001).

Many potential modes of compartmentalization have been considered, e.g., replication on the surface of mineral particles or within porous rocks, but biology makes universal use of bilayer lipid membranes for cellular compartmentalization. Whether there was at some stage a jump to this mode of organization, or whether this form of spatial localization came first, is unclear. In any case, this was a momentous step in the emergence of life, because cell membranes keep genomic molecules and their products physically together so that the genomic molecules can reap the benefits of any useful functions that they encode. At this level, the amazing ability of natural selection to generate diverse and highly adapted new life-forms came fully into play. In summary, just as the history of prebiotic chemistry involved a series of stages of increasing molecular complexity, so we imagine the origin of cellular life as the culmination of a series of stages of increasingly complex organization at the molecular and supramolecular levels, with the final stage being a recognizably biological structure capable of open-ended Darwinian evolution (Fig. 1). In this chapter, we refer to a cell-like structure with the as yet unrealized potential to evolve useful functions as a protocell; once the evolution of sequences that encode useful functions has occurred, we refer to such a structure simply as a cell.

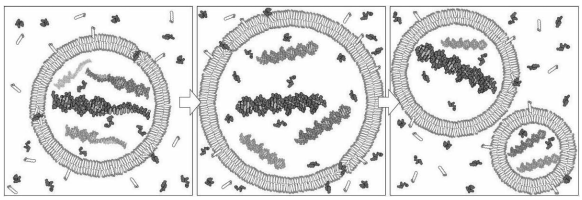

Figure 1. Schematic model of a protocell. A replicating vesicle enables spatial localization, and a replicating genome encodes heritable information. A complex environment provides nucleotides, lipids, and various sources of energy, including mechanical energy for division, chemical energy for nucleotide activation, and phase transfer and osmotic gradient energy for growth. (Reprinted, with permission, from Mansy et al. 2008 [Nature Publishing Group].)

PROTOCELL SYNTHESIS IN THE LABORATORY AND ON THE EARLY EARTH

We expect that efforts to synthesize model protocells in the laboratory will provide useful clues to the kinds of molecules and the nature of the physical environments that may have conspired to generate protocells on the early earth. Such efforts build on decades of pioneering work in prebiotic chemistry (Orgel 2004), the self-assembly and replication of membrane vesicles (Hanczyc and Szostak 2004), the nature of potential genetic polymers (Eschenmoser 1999), and the nonenzymatic template-directed copying of nucleic acid sequences (Joyce and Orgel 2006). Although ultimately interested in plausible scenarios for the origin of life, we have chosen to avoid, for now, the real or imagined constraints of prebiotic chemistry. Our immediate goal is simply to demonstrate the possibility of purely physicochemical replication schemes for model protocell membranes and genetic polymers and to show that these can be mutually compatible. Once such a system is devised, however artificial, we suspect that it will become much easier to construct related systems that are more realistic analogs of potential early earth systems.

NUCLEIC ACID REPLICATION

The initial polymerization of nucleotides into more or less random sequence polynucleotides has been shown to occur in at least two quite different ways: by local concentration effects arising from absorption to clay minerals (Ferris et al. 1996) or by freezing to eutectic conditions (Kanavarioti et al. 2001). The influence of minerals, such as montmorillonite, is particularly interesting because montmorillonite can catalyze both nucleic acid polymerization and membrane formation, thus bringing nucleic acid polymers and vesicle membranes together (Hanczyc et al. 2003). Although vesicles will encapsulate polynucleotide strands by spontaneous self-assembly around dissolved strands, the mineral-directed assembly and encapsulation of polynucleotides may significantly enhance the efficiency of this process. A recently described alternative is the simultaneous concentration of dilute fatty acids and nucleic acids by thermophoresis in thin channels such as those found in hydrothermal vents (Baaske et al. 2007), which leads to the assembly of vesicles containing encapsulated nucleic acids (Budin et al. 2009).

NONENZYMATIC TEMPLATE COPYING

Vesicle-encapsulated polynucleotides must replicate to be maintained during subsequent generations of vesicle growth and division; the ability of vesicles to acquire nucleotides from the environment provides a path for fueling template-dependent nucleic acid replication reactions inside vesicles. For replication reactions to occur without the aid of sophisticated catalysts such as protein enzymes or ribozymes, as may have occurred early in the origin of life, and as we wish to occur in our model protocells, it is necessary to consider modifications of standard nucleotide chemistry. For example, it has long been known that the substitution of imidazole for the pyrophosphate leaving group increases reactivity considerably, thereby allowing template-directed polymerization to occur spontaneously (Lohrmann and Orgel 1973, 1976). However, these more reactive nucleoside phosphorimidazolides are also subject to faster hydrolysis and cyclization, which compete with polymerization. Further increases in reactivity (without a corresponding increase in hydrolysis) can be obtained by substituting the hydroxyl nucleophile of the nucleotide with a better nucleophile, such as an amino group (Zielinski and Orgel 1985; Tohidi et al. 1987). The resulting phosphoramidate-linked polynucleotides are quite similar to the corresponding phosphodiester-linked polynucleotides. The template-directed polymerization of 3′-amino ribonucleoside phosphorimidazolides was initially examined by Orgel and colleagues (Lohrmann and Orgel 1976), but this line of inquiry was not pursued, apparently due to concerns that amino nucleotides were not prebiotically realistic. We have chosen to investigate a series of phosphoramidate polynucleotides (Fig. 2) with the goal of identifying a nucleic acid system capable of complete cycles of self-replication. Such a self-replicating nucleic acid would allow for the synthesis of interesting laboratory protocell models and might also provide clues to the nature of primitive chemically replicating genetic polymers on the early earth.

We have recently described the synthesis and base-pairing properties of the phosphoramidate version of glycerol nucleic acid (GNA) (Chen et al. 2009). Not surprisingly, this system is problematic due to the extremely rapid cyclization of its activated nucleotide monomers. However, we were able to circumvent this problem by synthesizing activated dinucleotides, which were in turn able to assemble into longer oligonucleotides by template-directed ligation. These experiments highlight the importance of a sterically constrained sugar moiety in a nucleotide monomer, so that the reactive nucleophile and leaving group are physically held apart from each other and cyclization cannot occur. One such system, which we have begun to study, is based on 2′-amino dideoxyribonu-

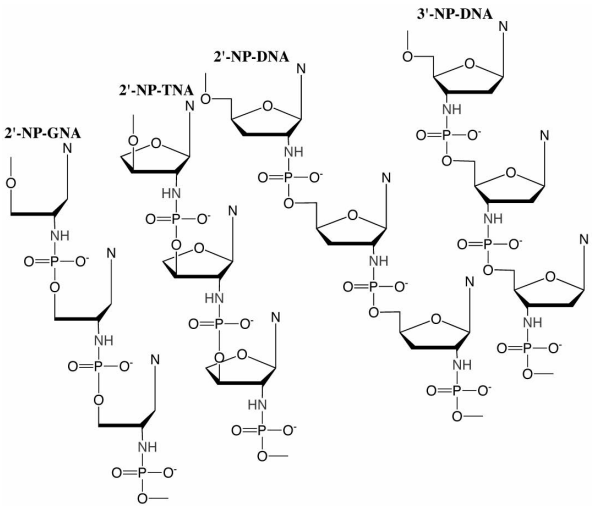

Figure 2. Phosphoramidate-linked nucleic acids. (*Left to right*) Phosphoramidate-linked glycerol nucleic acid (NP-GNA), threose nucleic acid (NP-TNA), 2′-5′-phosphoramidate DNA (2′-5′-NP-DNA), and 3′-5′-phosphoramidate DNA (3′-5′-NP-DNA).

cleotides, which cannot cyclize. Preliminary experiments showed that 2′-amino dideoxyguanosine, activated as the 5′-phophorimidazolide, was able to rapidly polymerize on an oligo(dC) template (Mansy et al. 2008). We are continuing to explore the potential of this promising system as a sequence-general, chemically replicating genetic polymer.

NUTRIENT ACQUISITION BY PROTOCELLS

Modern cells are highly organized structures that interact extensively with their surroundings through the selective absorption and release of chemicals. In the absence of protein transport machinery, protocells would have had to rely on the selective permeability of their membranes for nutrient uptake and waste release. Because membrane permeability properties dictate which molecules are available for protometabolic processes, it is important to evaluate the similarities and differences between model protocell membranes, composed of simple, prebiotically plausible amphiphiles such as fatty acids and related molecules, and contemporary biological membranes, which are composed of more complex amphiphiles such as phospholipids and sterols. In general, it appears that permeabilities are similar for small, uncharged molecules, whereas large differences are observed for ionic solutes (Hargreaves and Deamer 1978; Chen et al. 2005; Sacerdote and Szostak 2005).

Permeability of Membranes to Sugars and Nucleotides

Fatty acid membranes are similar to phospholipid membranes in that they are both permeable to and can discriminate between small uncharged molecules (Hargreaves and Deamer 1978; Sacerdote and Szostak 2005). Differences in permeation rates among small neutral solutes depend strongly on hydrophobicity and molecular size (Gerasimov et al. 1996; Kleinzeller 1999). A particularly interesting example is the permeability of ribose in comparison with its diastereomers. Despite having equal molecular weight and number of hydroxyl groups, the permeability coefficient of ribose is fivefold larger than that of its diastereomers (Sacerdote and Szostak 2005). Recent molecular dynamics simulations suggest that the greater permeability of ribose reflects its internally satisfied H-bonding interactions and consequently less extensive water–solute interactions (Wei and Pohorille 2009). Membranes composed of lipids that flip quickly and slowly have similar permeabilities, but increased fluidity does correlate with increased rates of permeation without loss of selectivity for ribose (Sacerdote and Szostak 2005; Mansy et al. 2008). The selective permeability of model prebiotic membranes for ribose suggests an early kinetic advantage of ribose over other five-carbon sugars. The observed preference for ribose could have provided an advantage for a protocell that used ribose (as opposed to any of its diastereomers) to make nucleotides.

An important difference between fatty acid and phospholipid membranes is that phospholipid membranes are generally impermeable to charged solutes, whereas fatty acid membranes are permeable. Differences in solute permeability that depend on the characteristics of individual fatty acid molecules have been studied using phospholipid membranes that contain trace amounts of fatty acids (Kamp and Hamilton 1992). The presence of low concentrations of fatty acid renders phospholipid membranes permeable to protons and metal ions. The data are consistent with a carrier mechanism in which carboxylate head groups, neutralized by protonation or complexation with metal, flip from one leaflet to another and then release their solute (Kamp and Hamilton 1992). Subsequent studies of monovalent and divalent cation permeability of membranes composed entirely of fatty acids are mechanistically consistent with these earlier studies on mixed phospholipids–fatty acid systems. However, because more exploitable solute interaction sites exist and because lipid flipping dynamics are faster in pure fatty acid membranes, cation fluxes are orders of magnitude higher than that for mixed phospholipids–fatty acid membranes (Chen and Szostak 2004).

In addition to small cations, larger charged organic molecules, such as nucleotides, can traverse fatty acid, but not phospholipid, membranes (Chen et al. 2005; Mansy et al. 2008). At slightly alkaline pH, nucleotide monophosphates have a charge of 2–, which greatly decreases their ability to cross hydrophobic barriers. However, complexation with Mg^{++} significantly increases permeability. The importance of charge neutralization is exemplified by comparison between AMP and ADP permeabilities. Although ADP is more highly charged, it also has a higher affinity for Mg^{++} (Khalil 2000) and so it is more easily neutralized. Thus, in the presence of Mg^{++}, ADP crosses fatty acid membranes more quickly than AMP (Mansy et al. 2008). Although the mechanism of nucleotide permeation is not known, the difference in permeation through fatty acid membranes versus phospholipid membranes is consistent with a dependence on lipid dynamics, perhaps via a mechanism similar to the

carrier mechanism described above for monovalent cations. It is striking that a charged and relatively large molecule, such as a nucleotide, can pass through fatty acid membranes, whereas larger polymers, such as proteins and nucleic acids, are retained. Large nonspecific pores must not be present in fatty acid membranes, because such pores would not be able to discriminate between mononucleotides and polymers.

Heterogeneous Membrane Composition

Primitive cell membranes must have been composed of mixtures of lipids, and the properties of such membranes can be quite different from those of membranes assembled from a single component (Mansy and Szostak 2008). Many of the permeability changes observed for mixed lipid membranes arise from decreased acyl-chain packing, and thus decreased van der Waals interactions. For example, permeability is increased by adding branched isoprenoid membrane components. Similarly, large bulky head groups result in a separation of acyl chains and thus decreased acyl-chain packing and increased solute permeability. The addition of 33 mol% of the glycerol ester of myristoleic acid (GMM) to a myristoleic acid (MA) membrane increases the permeability of the membrane to nucleotides by fivefold. However, it should be noted that head group influences may be much more complex than simple steric and shape effects. For example, charge repulsion between lipid head groups, solute–head group interactions, and the influences of the polarity of the head group on lipid flipping dynamics all affect the structure and permeability properties of membranes (Fig. 3). In addition, solute-lipid interactions could increase local solute concentrations thereby increasing permeation rates regardless of the mechanism invoked.

Heterotrophy

The permeability properties of primitive membranes are an important consideration for models of the nature of early cells. Highly impermeable membranes would have allowed for the retention of internally synthesized small molecules, but they would have prevented the absorption of nutrients from the environment as well as the release of chemical wastes. For such a system to survive, mechanisms for the catalysis of internal metabolic reactions, and for the replication of the catalysts, must have been present from the beginning. Thus far, little experimental progress has been made in creating laboratory models of such autotrophic protocells. Conversely, prebiotically plausible lipids self-assemble into semipermeable compartments capable of acquiring complex chemical nutrients without the aid of specific membrane transporters and without any danger of losing their genetic heritage. The resulting vesicle system lays the foundation for the construction of a laboratory model of a heterotrophic protocell capable of Darwinian evolution. However, for such a system to be realized, a full replication cycle including compartment growth and division, and nucleic acid replication, must be achieved.

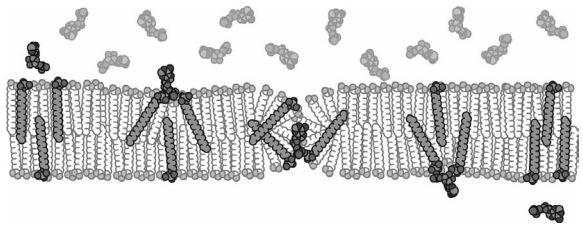

Figure 3. Model for spontaneous membrane permeation by charged solutes. Solute molecules form transient complexes with membrane amphiphiles through polar interactions with head groups and nonpolar interactions with acyl chains. Inversion across the membrane is followed by solute release. (Adapted, with permission, from Mansy et al. 2008 [Nature Publishing Group.])

TEMPLATE COPYING INSIDE VESICLES

The ability of fatty acid vesicles to passively absorb nucleotides coupled with the existence of an efficient nonenzymatic model of template-directed nucleotide polymerization allowed us to explore a simple protocell model involving nucleic acid replication inside a membrane vesicle. We were able to show that with only four components, excluding buffer and salts, a model protocell can be assembled that is capable of nutrient acquisition and internal nucleic acid copying (Mansy et al. 2008). The four components included two amphiphiles (the fatty acid myristoleic acid and its glycerol ester) and two oligonucleotides (a DNA primer and a DNA template). The fatty acids were used to prepare small unilamellar vesicles, which encapsulated the primer–template complex. When activated nucleotides were added to the outside of the vesicles, they diffused to the vesicle interior and polymerized by template-directed primer extension. The primer-extension reaction proceeded more slowly than analogous solution reactions, due to the time required for nucleotide permeation across the membrane but nevertheless progressed to completion in about 24 h.

Continued cycles of nucleic acid replication would result in increased internal osmotic pressure, because the polymer strands are trapped inside the vesicle, whereas the monomers can equilibrate between the internal compartment and external environment. This increased internal osmotic pressure can drive vesicle growth (Chen et al. 2004) at the expense of neighboring vesicles with less internal nucleic acid and thus a lower osmotic pressure. In effect, activated nucleotides can fuel both nucleic acid copying and the growth of fatty acid vesicles. Because this growth results from competition between vesicles for a limiting resource (fatty acids), any sequences that favored faster replication would come to dominate the population. This system is remarkable in that it suggests that it may be possible to create, from a relatively small number of components, a system that is capable of Darwinian evolution.

STRAND SEPARATION

Complete nucleic acid replication requires cycles of template copying followed by strand separation. Assuming

that some genetic polymer will be found that is capable of rapid and accurate strand copying, a mechanism for strand separation would then be required to complete the replication cycle. Strand separation can be achieved by a variety of means, including enzymatic, chemical, and thermal processes, but by far the simplest means of obtaining cycles of strand separation is through thermal fluctuations, as commonly used today in polymerase chain reaction (PCR). Indeed, the reconstitution of PCR with *Taq* polymerase (Oberholzer et al. 1995) inside impermeable phospholipid vesicles shows that strand separation can be thermally driven inside vesicles, as long as those vesicles are sufficiently stable and do not release their contents at high temperature.

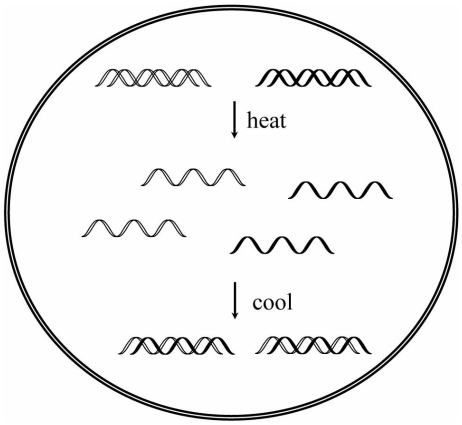

Figure 4. Strand separation inside vesicles. (Black lines) DNA strands labeled with donor and quencher dyes. When annealed to each other, fluorescence is low. (Open lines) Unlabeled DNA strands. Following strand separation and reannealing, the donor and quencher oligonucleotides are separated, resulting in a high fluorescence signal.

Thermal Stability of Fatty Acid Vesicles

Fatty-acid-based vesicles are in general more delicate than phospholipid vesicles and are disrupted by conditions such as high ionic strength, the presence of divalent cations, and extremes of pH that do not affect phospholipid vesicles (Monnard et al. 2002). We were therefore surprised to observe that fatty acid vesicles can be quite thermally stable, depending on their composition (Mansy and Szostak 2008). Adding fatty alcohols or fatty acid glycerol esters to pure fatty acid vesicles can result in dramatic stabilization. For example, myristoleic acid membranes will retain encapsulated DNA for 1 h at a maximum temperature of ~50°C, whereas membranes containing monomyristolein (GMM) were stable for 1 h to 100°C. Less stable membrane compositions, such as decanoic acid vesicles, also retained encapsulated DNA at 100°C, albeit for much shorter periods of time. In general, decreasing acyl-chain length or disrupting acyl-chain packing through branching results in decreased thermal stability.

Duplex Melting Inside Fatty-acid-based Vesicles

The surprising thermal stability of fatty-acid-based vesicles suggested that the strands of duplex nucleic acids could be thermally separated inside such vesicles without the loss of genetic material to the external environment. We were able to monitor thermal DNA melting and annealing by fluorescence spectroscopy of encapsulated duplex DNA, of which a trace amount was labeled with a fluorescent dye on one oligonucleotide and a quencher on the other; in this state, the dye fluorescence was largely quenched (Fig. 4) (Mansy and Szostak 2008). However, after thermal denaturation and reannealing, the dye and quencher were randomly distributed between separate duplexes, leading to a greatly increased fluorescent signal. We were also able to show that no DNA leaked out of the vesicles during the high-temperature treatment that led to DNA melting and strand separation.

What is the most plausible source of temperature fluctuations on the early earth that could have driven strand separation? Although day–night cycles are frequently invoked, diurnal temperature fluctuations on the modern earth are generally small, suggesting that sufficient warming to cause strand separation would require an average ambient temperature close to the melting point of the nucleic acid duplex. Because template copying chemistry generally proceeds optimally at low temperatures, this scenario is problematic. An interesting alternative scenario involves geothermal heating near hydrothermal vents or hot springs. In a cold environment, ponds might be partially covered with ice while being locally heated by hot rocks, which would induce convection currents that would result in entrained particles being occasionally briefly heated and then rapidly cooled. Laboratory models of such convection currents are capable of mediating PCR by carrying nucleic acids through regions of high and low temperatures to melt and anneal nucleic acids, respectively (Krishnan et al. 2002; Braun et al. 2003). Similar geothermally driven temperature cycles on the early earth may have been responsible for duplex melting and strand separation inside of semipermeable vesicles.

Vesicle Permeability at High Temperatures

The permeability of fatty-acid-based membranes to nucleotides is dramatically enhanced at high temperatures. Nucleotide equilibration across fatty acid membranes is less than half complete after 24 h at 23°C for 100-nm-diameter unilamellar vesicles, whereas at 90°C, the reaction is complete in <10 min (Mansy and Szostak 2008). Permeability enhancement at elevated temperatures complements thermally driven cycles of nucleic acid replication because the acquisition of fresh nucleotides and the dissipation of waste material can occur concurrently with strand separation. Thus far, the myristoleic acid:GMM model membrane system is the system most amenable to the construction of a model protocell, because these vesicles are both highly permeable and highly thermostable while forming at a relatively low amphiphile concentration of ~1 mM. A more prebiotically reasonable membrane composition consisting of decanoic acid mixed with decanol and a decanoate-glyc-

erol ester requires a higher amphiphile concentration to form vesicles (~20 mM) and can only withstand brief high-temperature excursions. Because the esterification of glycerol to a fatty acid can occur under model prebiotic conditions by dehydration condensation (Apel and Deamer 2005), the first step in constructing phospholipids from fatty acids would have been both chemically simple and advantageous.

COMPARTMENT DIVISION

An essential aspect of the design of a model protocell is the nature of the compartment division process. Until recently, no robust but simple cell division mechanism has been described. Modern cells rely on an intricate set of cytoskeletal proteins that coordinate membrane division with cell wall growth and nucleic acid partitioning (Margolin 2000). However, studies of pure phospholipid membranes demonstrate that budding and division can be induced with thermal or osmotic fluctuations without the aid of proteins (Hanczyc and Szostak 2004). The relevant physical forces arise from membrane asymmetries, such as differences in leaflet composition or area, the presence of phase-separated lipid domains, or changes in the ratios of surface area to volume. These processes are important in that they demonstrate the ability of vesicles to divide in the absence of protein machinery, but they cannot form the basis of a cell cycle because they rely on asymmetries that are lost upon division.

A crude but effective way of forcing vesicles to divide is by extrusion through small pores. Despite the very high shear stresses imposed by extrusion, only 30%–40% of encapsulated molecules are lost to the environment during division (Hanczyc et al. 2003). This is little more than the volume decrease that must occur upon division of a spherical vesicle into two daughter vesicles with the same total surface area. The retention of contents during extrusion suggests that division occurs by the pinching off of the daughter vesicles, as opposed to the disruption of the parental vesicle and the resealing of smaller membrane fragments. Because the exposure of acyl chains to aqueous solution is highly energetically unfavorable, the membrane resists tearing and any transiently induced pore rapidly seals. Although compartment division can be induced by extrusion, we have argued on hydrodynamic grounds that an analogous process is unlikely to have operated on the early earth (Zhu and Szostak 2009). However, the laboratory demonstration of vesicle extrusion as a division mechanism allowed us to demonstrate a cyclical process of growth via feeding with micelles followed by extrusion-induced division (Hanczyc et al. 2003). Such a cycle could be used as the basis of a laboratory model of a protocell, but a more efficient system that avoided the loss of a substantial fraction of the protocell contents during each division cycle would be highly desirable.

Most studies of the growth and division of fatty acid vesicles have focused on the behavior of small unilamellar vesicles, which are relatively homogeneous. However, spontaneous vesicle formation, either by rehydration of dried films or by the acidification of alkaline micelles, generates a heterogeneous mixture of vesicles of varying lamellarity and a wide size distribution. Recent fluorescence microscopy studies of large multilamellar vesicles revealed a surprising mode of vesicle growth and division (Fig. 5) (Zhu and Szostak 2009). Following the addition of excess micelles, multilamellar vesicles grow by forming a thin tail-like protrusion; during ~30 min, the thin protrusion elongates and thickens until the entire original vesicle is subsumed into the resulting long filamentous vesicle. This filamentous vesicle is quite fragile and divides into smaller daughter vesicles upon gentle agitation, with no loss of contents to the environment (Zhu and Szostak 2009). The daughter vesicles that form by fragmentation of the filamentous vesicle are still multilamellar, and they can grow again with the addition of more fatty acids in the form of micelles. The precise mechanism of division remains unclear, but highly elongated phospholipid vesicle structures are known to spontaneously divide into many daughter vesicles through the pearling instability (Bar-Ziv and Moses 1994), and the division of filamentous fatty acid vesicles may also proceed through the pearling instability. Growth and division through a filamentous intermediate state were observed for a wide range of membrane compositions, vesicle sizes, and environmental conditions. Two critical factors contribute to this growth mode: a transient imbalance between surface area and volume growth, and the multilamellar nature of the vesicles. In the presence of a highly permeable buffer such as ammonium acetate, volume growth is not limited by slow solute equilibration; as a result, the outer membrane of the initial vesicle swells up spherically, and no filamentous protrusion is formed. In contrast to multilamellar vesicles, large unilamellar vesicles grow more symmetrically into elongated structures but never form a thin initial protuberance. In addition, after growth, large unilamellar vesicles are disrupted by gentle agitation and lose much of their contents to the environment. Growth and division via a filamentous intermediate structure is appealing because it occurs with vesicles that form spontaneously, and therefore appears to be prebiotically plausible. In addition, growth and division are mechanistically coupled because it is the pattern of growth that predisposes the vesicle to division (Zhu

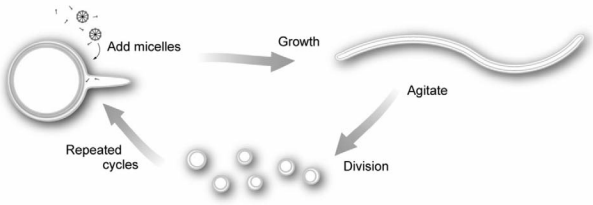

Figure 5. Cycles of vesicle growth and division. A spherical multilamellar vesicle grows after the addition of fatty acid micelles by the formation of a thin protuberance. This grows over time until the initially spherical vesicle transforms into filamentous vesicle. Gentle agitation leads to division into daughter vesicles, which in turn can grow and repeat the cycle. (Reprinted, with permission, from Zhu and Szostak 2009 [©American Chemical Society].)

and Szostak 2009). This vesicle growth–division cycle is very efficient at retaining encapsulated contents. The fact that multilamellar vesicles grow into filamentous vesicles that are predisposed to divide upon gentle agitation is a strong indication that essential life-like properties can emerge from simple chemical components in an appropriate physical environment.

CONCLUSIONS

Simple amphiphiles such as fatty acids and related molecules spontaneously assemble into membrane vesicles that have properties which are well-suited for models of primitive cells, in that they can grow by incorporating additional molecules, and they allow for the uptake of charged nutrients such as nucleotides from the environment. They are compatible with the chemistry of nucleic acid template copying, and we have been able to demonstrate such copying reactions using encapsulated templates. These primitive membranes are surprisingly thermostable, and they withstand the high temperatures required for strand separation of internal nucleic acid duplexes. Finally, multilamellar vesicles undergo a shape transformation, from spherical to filamentous, during growth, and the resulting filamentous vesicles are sufficiently fragile that they divide into daughter vesicles in response to gentle agitation.

Although this recent progress toward the synthesis of model protocells is quite encouraging, many challenges remain. Foremost among these is the design and synthesis of a genetic polymer capable of repeated cycles of chemical (i.e., nonenzymatic) replication; this is the central barrier to the synthesis of a complete protocell. Amino nucleotides, which polymerize into phosphoramidate-linked nucleic acids, are good candidates for the basis of such a chemical replication system, and we are currently exploring several such systems. A related and also potentially serious problem is strand reannealing, which competes with template copying. Because the reannealing of complementary strands is quite rapid, some means for slowing down reannealing to a timescale comparable to that of template copying must be identified. At this point, there do not appear to be major problems associated with the growth and division of model protocell membranes or with the integration of the protocell membrane with the internal nucleic-acid-copying chemistry. However, an important future goal is to identify simple means that will coordinate the replication of the nucleic acid genome with the replication of the membrane compartment.

Although our work has been directed toward the design and synthesis of a model protocell, our experiments do have implications for theories of the origin of life on the early earth. These implications stem primarily from the surprising physical properties of membranes composed of simple amphiphiles such as fatty acids and related molecules. These membranes seem to possess many of the properties that would be necessary for a primitive cellular system. However, as previously noted (Deamer 1997), fatty acid membranes are incompatible with the high salt and divalent cation concentrations of marine environments and can only exist in freshwater environments. The requirement for cycling between low and high temperatures for nucleic acid copying and strand separation strongly suggests that freshwater ponds or springs in a generally cold environment, locally heated by geothermal activity as in a volcanic region, would be an ideal incubator for the origin of life.

ACKNOWLEDGMENTS

We thank J. Schrum, T. Zhu, A. Ricardo, J. Chen, I. Budin, and the many other current and former members of the Szostak lab who have contributed to the work described in this chapter. We are also grateful to J. Iwasa for Figure 3. J.W.S. is an Investigator of the Howard Hughes Medical Institute. S.S.M. is supported by the Armenise-Harvard foundation. This work was supported by grants from the National Science Foundation (CHE 0434507) and the NASA Exobiology section (EXB02-0031-0018) and fellowships from the Harvard Origins Initiative.

REFERENCES

Apel CL, Deamer DW. 2005. The formation of glycerol monodecanoate by deydration/condensation reaction: Increasing the chemical complexity of amphiphiles on the early earth. *Orig Life Evol Biosph* **35:** 323–332.

Baaske P, Weinert FM, Duhr S, Lemke KH, Russel MJ, Braun D. 2007. Extreme accumulation of nucleotides in simulated hydrothermal pore systems. *Proc Natl Acad Sci* **104:** 9346–9351.

Bar-Ziv R, Moses E. 1994. Instability and "pearling" states produced in tubular membranes by competition of curvature and tension. *Phys Rev Lett* **73:** 1392–1395.

Braun D, Goddard NL, Libchaber A. 2003. Exponential DNA replication by laminar convection. *Phys Rev Lett* **91:** 158103.1–158103.4.

Budin I, Bruckner RJ, Szostak JW. 2009. Formation of protocell-like vesicles in a thermal diffusion column. *J Am Chem Soc* **131:** 9628–9629.

Chen IA, Szostak JW. 2004. Membrane growth can generate a transmembrane pH gradient in fatty acid vesicles. *Proc Natl Acad Sci* **101:** 7965–7970.

Chen IA, Roberts RW, Szostak JW. 2004. The emergence of competition between model protocells. *Science* **305:** 1474–1476.

Chen IA, Salehi-Ashtiani K, Szostak JW. 2005. RNA catalysis in model protocell vesicles. *J Am Chem Soc* **127:** 13213–13219.

Chen JJ, Cai X, Szostak JW. 2009. N2′ → P3′ phosphoramidate glycerol nucleic acid as a potential alternative genetic system. *J Am Chem Soc* **131:** 2119–2121.

Deamer DW. 1997. The first living systems: A bioenergetic perspective. *Microbiol Mol Biol Rev* **61:** 239–261.

Eschenmoser A. 1999. Chemical etiology of nucleic acid structure. *Science* **284:** 2118–2124.

Ferris JP, Hill AR, Liu R, Orgel LE. 1996. Synthesis of long prebiotic oligomers on mineral surfaces. *Nature* **381:** 59–61.

Gerasimov OV, Rui Y, Thompson TE. 1996. Triggered release from liposomes mediated by physically and chemically induced phase transitions. In *Vesicles* (ed. M Rosoff), pp. 679–746. Marcel Dekker, New York.

Hanczyc MM, Szostak JW. 2004. Replicating vesicles as models of primitive cell growth and division. *Curr Opin Chem Biol* **8:** 660–664.

Hanczyc MM, Fujikawa SM, Szostak JW. 2003. Experimental models of primitive cellular compartments: Encapsulation, growth, and division. *Science* **302:** 618–622.

Hargreaves WR, Deamer DW. 1978. Liposomes from ionic, single-chain amphiphiles. *Biochemistry* **17:** 3759–3768.

Joyce GF, Orgel LE. 2006. Progress toward understanding the origin of the RNA world. In *The RNA world* (ed. RF Gesteland et al.), pp. 23–56. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kamp F, Hamilton JA. 1992. pH gradients across phospholipid membranes caused by fast flip-flop of un-ionized fatty acids. *Proc Natl Acad Sci* **89:** 11367–11370.

Kanavarioti A, Monnard PA, Deamer DW. 2001. Eutectic phases in ice facilitate nonenzymatic nucleic acid synthesis. *Astrobiology* **1:** 271–281.

Khalil MM. 2000. Complexation equilibria and determination of stability constants of binary and ternary complexes with ribonucleotides (AMP, ADP, and ATP) and salicylhydroxamic acid as ligands. *J Chem Eng Data* **45:** 70–74.

Kleinzeller A. 1999. Charles Ernest Overton's concept of a cell membrane. In *Membrane permeability: 100 years since Ernest Overton* (ed. DW Deamer et al.), pp. 1–18. Academic, San Diego.

Krishnan M, Ugaz VM, Burns MA. 2002. PCR in a Rayleigh-Bénard convection cell. *Science* **298:** 793.

Lohrmann R, Orgel LE. 1973. Prebiotic activation processes. *Nature* **244:** 418–420.

Lohrmann R, Orgel LE. 1976. Template-directed synthesis of high molecular weight polynucleotide analogues. *Nature* **261:** 342–344.

Mansy SS, Szostak JW. 2008. Thermostability of model protocell membranes. *Proc Natl Acad Sci* **105:** 13351–13355.

Mansy SS, Schrum JP, Krishnamurthy M, Tobe S, Treco DA, Szostak JW. 2008. Replication of a genetic polymer inside of a model protocell. *Nature* **454:** 122–125.

Margolin W. 2000. Themes and variations in prokaryotic cell division. *FEMS Microbiol Rev* **24:** 531–548.

Monnard PA, Apel CL, Kanavarioti A, Deamer DW. 2002. Influence of ionic inorganic solutes on self-assembly and polymerization processes related to early forms of life: Implications for a prebiotic aqueous medium. *Astrobiology* **2:** 139–152.

Oberholzer T, Albrizio M, Luisi PL. 1995. Polymerase chain reaction in liposomes. *Chem Biol* **2:** 677–682.

Orgel LE. 2004. Prebiotic chemistry and the origin of the RNA world. *Crit Rev Biochem Mol Biol* **39:** 99–123.

Sacerdote MG, Szostak JW. 2005. Semipermeable lipid bilayers exhibit diastereoselectivity favoring ribose. *Proc Natl Acad Sci* **102:** 6004–6008.

Szostak JW, Bartel DP, Luisi PL. 2001. Synthesizing life. *Nature* **409:** 387–390.

Tohidi M, Zielinski WS, Chen CH, Orgel LE. 1987. Oligomerization of 3′-amino-3′ deoxyguanosine-5′ phosphorimidazolidate on a d(CpCpCpCpC) template. *J Mol Evol* **25:** 97–99.

Wei C, Pohorille A. 2009. Permeation of membranes by ribose and its diastereomers. *J Am Chem Soc* **131:** 10237–10245.

Zhu TF, Szostak JW. 2009. Coupled growth and division of model protocell membranes. *J Am Chem Soc* **131:** 5705–5713

Zielinski WS, Orgel LE. 1985. Oligomerization of activated derivatives of 3′-amino-3′-deoxyguanosine on poly(C) and poly(dC) templates. *Nucleic Acids Res* **13:** 2469–2484.