Nonreplicating Protocells

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CONSPECTUS

Prebiotic soup experiments have shown that the molecular building blocks of life can be built under prebiotically plausible conditions. From this starting point, researchers have launched continued studies of polymerization and explorations of the breadth of RNA function. Recently, effort has intensified to examine experimentally another stage of the origins of life: the assembly of the molecular parts into model protocells intended to represent the first primitive, cell-like systems to emerge on Earth.

Although it may not be possible to recreate the precise sequence of events that led to cellular life, laboratory experiments have begun to show what was and was not possible. Prebiotically plausible lipid vesicles form easily and have many properties that are conducive to cellular function. In addition to protecting nascent replicating genetic systems from parasitic sequences, vesicles facilitate evolution. The data thus far suggest that prebiotically plausible vesicles could have grown, divided, and promoted competition between distinct chemical systems. Most protocellular studies to date have probed the role of self-replication, one feature of extant life in the emergence of the first cellular system. Undoubtedly replicating systems were crucial for protocellular evolution, but other features of life must have been important as well. For example, life does not exist in isolation. A living system must cope with and adapt to environmental fluctuations to survive. The protocell must have generated some of these fluctuations because cellular activity necessarily modifies its surroundings by selectively absorbing nutrients and releasing unwanted molecules. It seems likely that life would have faced this challenge early and either emerged in dynamic locales that continuously regenerated conditions conducive to life or exploited mechanisms to physically move to new areas not depleted in resources. Further studies that explore non-replication-based aspects of the origins of life could reveal a more complete picture of the transition from prebiotic chemistry to early life.
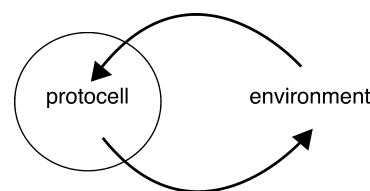
Living Cells Replicate

Several laboratories are attempting to build model protocellular systems in the laboratory to better understand how life could have emerged on prebiotic Earth, but it is unclear what exactly should be built. There are no agreed upon prebiotic conditions to begin with nor is it obvious which molecular building blocks were and were not available. We do not know the type of metabolism that was exploited by early life. Current knowledge does not even extend back to the last universal common ancestor, a phase of evolution significantly more complex than the earliest stages of protocellular development. Perhaps most frustratingly, we may not be able to recognize success if a laboratory made protocellular system were constructed that faithfully mimicked Earth's first cells.

Despite the numerous difficulties, significant steps forward in understanding the chemistry of the origins of life have occurred. The Miller−Urey experiments,[1] for example, demonstrated how carefully planned and executed laboratory research can give interpretable results with implications for the origins of life. In this case, the lesson was that simple molecules, such as water, methane, ammonia, and hydrogen, can react to form amino acids.[2] Over half a century later, research continues to be inspired by such prebiotic soup approaches. To date, prebiotically relevant mechanisms are available for the synthesis of nucleotides,[3] amino acids,[2] lipids,[4] and sugars.[5] Interestingly, analyses of meteorites confirm that the building blocks of life can be built abiotically.[6−8]

It was only natural that research would continue to build logically on the successes of prebiotic synthesis experiments. Once the building blocks were formed, they must have assembled in some way into functional polymers and higher order aggregate structures. Consistent with such expectations, several prebiotically plausible methods have been reported that concentrate and thus promote the
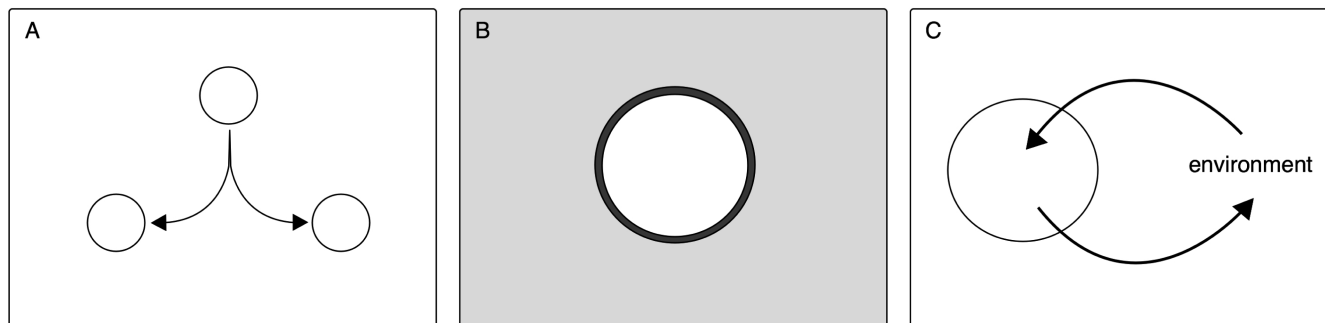
FIGURE 1. There are several features of life that could be mimicked chemically in the laboratory. For example, living systems replicate (A), have identity (B), and interact with the environment (C). Panel B empasizes the boundary structure (dark gray) between the inside of a (proto)cell (white) and the environment (light gray).

reactivity of monomeric, molecular building blocks. For example, mineral surfaces,[9,10] eutectic phases,[11] aerosols,[12] and convection and thermophoresis[13,14] all promote aggregation and thus reactivity. Since active sequences can emerge from pools of random polymers,[15,16] the narrative appears largely complete. Monomeric building blocks were synthesized from simple starting material that then polymerized into functional sequences that aggregated into Earth's first cells.

While the data are quite convincing, the way in which the data have been used has been narrow in scope. One reason may be the disproportionate interest in building self-replicating systems (Figure 1A). RNA selection and evolution experiments largely seek to produce a replicase, that is, an RNA molecule that functions as an RNA-dependent RNA polymerase.[17−19] Similarly, assembling components into model protocells has tended to focus on growing and dividing vesicles containing a replicating genome.[20−22] The reason for this replication bias is clear. All known living things have a genome, and, aside from evolutionary dead ends, all living systems replicate. However, by neglecting other features of life, we may be slowing progress in deciphering the origins of cellular life and missing opportunities to gain deeper insight into biology.

Building chemical systems under defined conditions that mimic shared features of life other than replication,[23] such as as the ability to modify the environment, to move, and to sense, respond, and adapt to stimuli, likely would give deeper insight into what was and was not possible during the transition from prebiotic to biotic systems. Thus far, much of the data acquired on model protocellular systems support the concept that the underlying organization of basic cellular function is due to fundamental chemical-physical forces.[24] In other words, many of the features of life can be mimicked chemically without exploiting

genetically encoded, complex, and prebiotically unrealistic protein machinery. Therefore, it is probable that the exploration of nonreplication features of life would contribute to elucidating potential chemical-physical paths toward the emergence of life on Earth.

Cells Have Identity

The first place to begin when building a protocell is to give the cell-like system identity. Since life is not in thermodynamic equilibrium with the environment, a separation between the two is required (Figure 1B). There are several ways to achieve segregation between protocells and the environment. For example, porous rock has been proposed as an attractive possibility for housing protocellular systems, since mineral surfaces can provide useful catalytic properties.[13,25] However, all extant life uses lipid defined membranes, and lipids form easily in aqueous solution. Further, the static confines of porous rock would likely inhibit progression toward more complex systems unless later developments gave rise to new mechanisms capable of moving the system into a compartment more amenable to growth and division, such as lipid vesicles. Finally, porous rock does not look like a cell. Morphology is not simply an aesthetic consideration, but instead influences how a chemical system physically experiences its environment. Protocells with lipid membranes do not suffer from such complications. In fact, the ease in which many lipids form vesicles suggests that vesicles existed on Earth before there was life.[26]

The identity provided by lipid membranes does not inhibit other lifelike processes. Rather than sealing off a chemical system from the surroundings, membranes allow for the selective acquisition, retention, and release of molecules without a dependence on complex, prebiotically unlikely macromolecules. Even subtle differences in the chemical characteristics of a molecule can result in dramatic
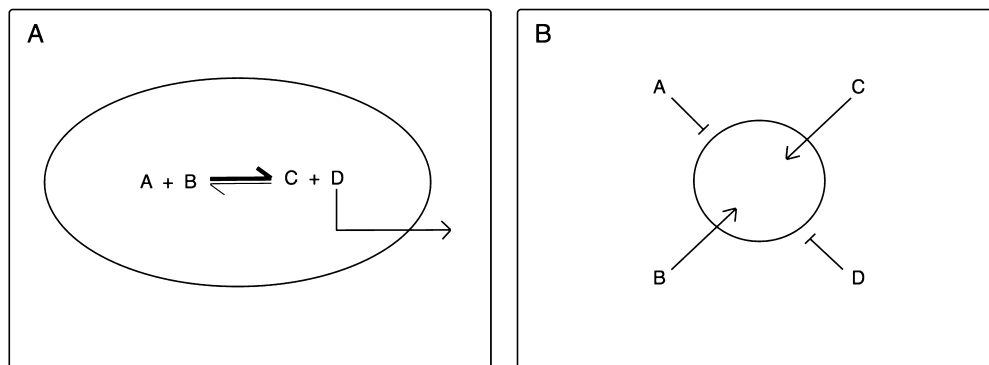
FIGURE 2. Permeability properties of vesicle membranes could have facilitated useful prebiotic chemistry. (A) A semipermeable membrane can help shift the equilibrium toward product formation, if one of the products (D) can cross the membrane. (B) Since some molecules are incapable of crossing a lipid membrane (A and D) while others readily permeate through membranes (B and C), a semipermeable membrane can control which molecules react within the vesicle.

changes in permeation rates across model protocell membranes.[27] Selective exchange between a protocell and the environment is not only important for the acquisition of nutrients, but also the ability to block the transport of some molecules allows for the establishment of concentration gradients, and thus for the storage of free energy. The generation of a concentration gradient could be achieved simply by the encapsulation of molecules incapable of passing through the membrane during vesicle formation followed by vesicle migration to a new environment devoid of the same molecule. Alternatively, the synthesis of molecules that cannot permeate the membrane from starting material that can cross the lipid boundary would result in a concentration gradient across the protocell membrane. The latter process was observed when nonenzymatic nucleic acid replication mechanisms were reconstituted inside of fatty acid vesicles in the sense that full-length product nucleic acid was not capable of leaking out into the extravesicular space.[28] Finally, proton gradients naturally form during vesicle growth of fatty acid membranes, which under some conditions can be maintained for prolonged periods of time.[29] Consequently, there is enough data now on model protocells to demonstrate that, rather than inhibiting lifelike processes, the identity provided by lipid membranes help create out of equilibrium systems with cell-like features.

The generation of a concentration gradient alone is not useful unless the free energy associated with the gradient can be coupled with other needed processes. Interestingly, such a coupling was observed when vesicles were loaded with nucleic acids, because the osmotic pressure resulting from the concentration gradient was capable of fueling the growth of the vesicle.[30] This suggests that once an encapsulated mechanism that allowed for the replication of nucleic

acids was established, vesicles with replicating nucleic acids naturally would grow and divide.[22] Other simpler routes toward the coupling of concentration gradients with useful chemistry likely existed. For example, contemporary metabolism exploits chemical transformations that under standard conditions are unfavorable, but are nevertheless favorable under cellular conditions. One of the several ways in which cells accomplish such a task is by exploiting mass action effects. Semipermeable membranes could have provided for a prebiotically accessible way to exploit mass action. For instance, an equilibrium between reactants A and B and products C and D that under standard conditions favored the formation of A and B could be altered by the presence of a semipermeable membrane. If the reaction took place inside of a vesicle and if one of the products were capable of crossing the membrane (e.g., product D), then an increase in the production of C and D would become favorable (Figure 2A). Since degradation and polymerization are competing processes, mass action effects within semipermeable membranes could have helped shift the balance toward polymerization, if the proper permeability properties existed.

The identity provided to chemical systems by vesicles could have facilitated prebiotic processes in at least one more way. A commonly encountered prebiotic chemistry problem is the numerous reaction pathways that can be taken by reactive molecules. Uncontrolled chemical reactions typically give a large diversity of products, resulting in insoluble tar. Without a mechanism to limit the available paths in which molecules can react, it seems unlikely that exploitable molecules could have reached a sufficient concentration for use by protocellular systems. As previously noted by Copley et al., one way in which a few pathways could have emerged from the many chemical possibilities

would have been if catalysts were present that helped kinetically funnel reactions down specific paths.[31] Such a directed flux of molecules would have restricted the range of options and facilitated the accumulation of specific end products. Although interesting examples have been reported,[32] none have probed the potential benefit of including vesicles in the reaction. Since the semipermeable properties of membranes are effective in filtering out many molecules, the range of possible chemical reactions within the interior of a vesicle compartment would have been restricted (Figure 2B). Perhaps the combined effects of catalysts and semipermeable membranes impacted the selection of which chemical reactions would constitute protocellular and thus eventually cellular metabolism.

Life and the Environment Both Influence Each Other

Although a protocell must be distinct from the environment, extracellular factors are indispensible for cellular function. Both the environment and the protocellular system must be considered at some level as a whole. Indeed, the vesicle compartment itself requires specific chemical conditions to form. Extremes in pH and salinity, for example, rapidly degrade vesicles composed of fatty acid membranes. However, the sensitivity of model protocell membranes also permits the environment to exert some control on the behavior of the system. In the absence of highly evolved machinery, protocells would have had to rely on such environmental forces to advance cell-like functions. For instance, the mixing of fatty acid solutions of different pH could have promoted the growth and division of vesicles on prebiotic Earth.[33,34] Similarly, since prebiotically plausible vesicles are temperature stable,[35,36] temperature fluctuations could have assisted nucleic acid replication.[37] In short, there are many ways in which the environment could have driven protocellular activity.

If the environment significantly affected protocells, could protocells also have affected the environment? The history of life on Earth seems to suggest that Earth's first cells would have influenced early Earth. The fact that the Earth went from being anaerobic to aerobic due to the activity of life illustrates the impact that life can have on the environment. Although protocellular activity likely had a more limited effect on the environment, the simple existence of protocells would have meant that the environment would have been used as a source of chemical nutrients and as a space to dissipate unwanted chemical species that interfere with
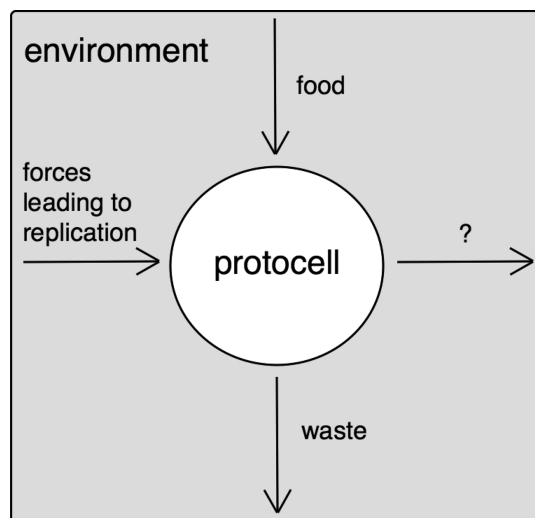

FIGURE 3. Potential interactions between a protocell and the environment. A protocell consumes molecules from the environment and releases waste molecules into the environment. Both processes change the chemical composition of the surroundings. Environmental fluctuations impact the behavior of the protocell, potentially leading to protocell replication. There likely were additional ways in which protocells impacted the environment.

needs of the protocell (Figure 3). In other words, protocells would have changed the composition of their environment by selectively sequestering, transforming, and releasing specific molecules. However, the scale of such an effect, particularly during the earliest stages of evolution, is unclear.

Nevertheless, protocells consumed molecules found in the environment. To avoid death, protocells would have had to find ways to continually find more food to substitute for the molecules that previously were consumed. A simple solution would be if the protocell formed in an environment that continuously produced the needed nutrients and flushed away protocellular waste, as has been proposed for regions similar to modern day black smokers and hydrothermal vents.[38] In such an environment, the protocell would not have required a mechanism to cope with depleting supplies, thus giving the system time to develop in complexity. However, the assembly of polymers from monomers would be challenging under hydrothermal vent conditions. While such a scenario is attractive due to its simplicity, other more active mechanisms are equally simple. For example, Dobson et al. proposed that aerosols formed at the air−water interface could have carried protocells to new locations.[12] An attractive feature of this proposal is that the entry of aerosol particles into a lipid solution could conceivable transform the aerosol particles into vesicles, thereby generating an aerosol−vesicle cycle capable of exploring varied regions of prebiotic Earth. An even more

active search for food was described by Hanczyc et al.[39] Their work showed that physical forces can propel oil based chemical systems toward nutrients and away from exhausted supplies. Although the oil based systems are far from what is typically considered lifelike, the experiments demonstrate that even a few types of chemicals can begin to display a type of sense−response phenotype.

The ability to sense and respond to the environment is a shared feature of life that likely was established early in evolutionary history. Nonetheless, relatively little effort was expended in exploring how prebiotic mechanisms could have allowed protocells to adapt to fluctuating conditions. The neglect of the topic is strange, because synthetic biology research, a field that shares many of the same goals as origins of life studies, is heavily invested in engineering sense−response pathways.[40,41] The search for food is only one example of a problem that could be better addressed by a protocell with an appropriate sense−response system. A protocell would have faced many environmental fluctuations with potentially destructive effects. The fact that some of the detrimental environmental changes would have resulted from the activity of the protocell itself suggests that life must have faced this challenge early.

A protocellular system that senses, responds, and contributes to changing the environment blurs the distinction between the protocell and the environment. The chemical system of the protocell could be viewed as a subset of a larger chemical system that cycles through the protocell and the environment. The resulting dynamic relationship between the protocell and the environment and how the two evolved with each other over time to shape contemporary life provides an interesting, non-replication centric perspective from which to probe the origins of life.

Conclusion

Progress in prebiotic chemistry has in many ways continued along the same trajectory from the contributions of Miller−Urey in the 1950s to now. The underlying theme can be captured by the most invoked definition of life, that is, a self-sustained chemical system capable of undergoing Darwinian evolution.[42] We have benefited greatly and will continue to learn much from experiments geared toward building prebiotic models of self-replicating systems. However, a more complete picture of the early stages of life would likely emerge by delineating the fundamental forces behind other shared features of life, such as the ability to sense, respond, adapt, and evolve with the environment.

BIOGRAPHICAL INFORMATION

Cristina Del Bianco was born in Florence, Italy. She received a *laurea* in chemistry from the University of Rome *La Sapienza*. Her doctoral work applied paramagnetic NMR techniques to the study of metalloproteins with C. Luchinat at the University of Florence. After completing postdoctoral research at Harvard Medical School in which she investigated the biochemistry of Notch signaling with S. C. Blacklow, she moved to the University of Trento. She is currently leading Trento's first iGEM team.

Sheref S. Mansy was born in Oregon and received his undergraduate and graduate degrees from Ohio State University. He then worked on building model protocellular systems in the Szostak laboratory at Massachusetts General Hospital. Upon receiving a career development award from the Armenise-Harvard foundation, he moved to the University of Trento. His laboratory builds cell-like systems. He is a 2012 TEDGlobal fellow.

FOOTNOTES

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