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Primer

Protocells

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The cell is the basic unit of life as we know it. But are cells truly necessary for life? To probe this question, one may start with NASA's 'working definition' of life that is now widely used: a self-sustaining chemical system capable of Darwinian evolution. The term 'self-sustaining' encompasses many interesting aspects, such as metabolism and environmental driving forces (e.g., diurnal cycling). This aside, an information-bearing molecular system that replicates should meet this definition of life, since errors (mutations) are an inevitable feature of real chemical systems. Artificial molecular replicators have been made from RNA, DNA, peptides and even small molecules, albeit with varying potential for variation and evolution.

Considering life on Earth, it is parsimonious, experimentally expedient and increasingly plausible to consider RNA as the basis for a simple kind of life, given its remarkable capacity to both store information and serve as a biocatalyst. Imagine that writing about a horse would actually create a horse from those letters (Figure 1) — such is the wondrous nature of RNA. The idea that life began with RNA (the 'RNA World') has been extensively debated and reviewed. The great scientific utility of this concept is to provide a concrete, experimentally attainable vision of a primitive life form: a system of RNAs with catalytic activities (ribozymes) that enable sequence replication and any necessary metabolic functions. The complexity needed in such a system depends on the richness of the environment provided. For example, an RNA-dependent RNA polymerase (an RNA replicase) might fulfill NASA's definition of life if the environment could supply nutrients such as nucleotides, primers and Mg^{2+}. More pointedly, a couplet of mutually dependent RNA ligases, each catalyzing formation of the other, undergoes Darwinian evolution as demonstrated by Lincoln and Joyce in 2009. So, why do we need cells?

There are at least two fundamental reasons why cells are important to life. First, they serve as a semi-permeable barrier for nutrients and waste products while keeping the molecular genome and the metabolism of an organism linked (Figure 2). To understand the second reason, consider an RNA replicase whose sequence arises by chance (e.g., by non-enzymatic polymerization) in an environment conducive to self-replication. If the replicase diffuses freely in solution, it encounters other RNAs and copies them, but no other molecules copy the replicase. There may be some non-specific copying of the replicase, but its activity actually creates a selection pressure for good templates, not good catalysts. Such situations result in parasitic sequences overwhelming the selection (Figure 2), and the altruistic replicase disappears.

The first in vitro molecular incarnation of such evolution occurred in Sol Spiegelman's famous Qβ replicase experiment. The Qβ replicase is a protein enzyme (from phage) that replicates the RNA that encodes it. When provided with an in vitro environment enabling replication and translation, truncated replicase mutants that lacked enzymatic activity but served as preferred templates arose and accumulated. Eventually, Spiegelman's 'monsters', unable to replicate themselves, drove the system to a halt. This is indeed Darwinian evolution. But it is not particularly interesting on its own. Biological evolution on Earth has exhibited tremendous creativity and innovation, which can be termed 'open-ended evolution'. A minimal requirement for such evolution is to prevent parasites from crashing the system. There are many mechanisms that select for cooperative traits, and one such trait that is available to even simple molecular systems is compartmentalization. By physically separating different genomes from each other, cells create a new unit of selection. Cells containing parasites

would not be able to replicate, allowing cells with replicases and other cooperative phenotypes to flourish (Figure 2). These systems have been studied both theoretically, and increasingly, experimentally.

Protocells as an experimental model

For such very primitive cells, usually called 'protocells', the compartments should be simple enough to self-assemble. Indeed, compartments can be surprisingly easy to form. A notable example is David Deamer's resuspension of organic extract from the Murchison meteorite, a carbonaceous chondrite that fell 50 years ago in Australia and is still being actively analyzed. The organic matter, when resuspended in water, assembled spontaneously into vesicular structures that resemble cells. The Murchison meteorite is rich in organics and contains lipids such as fatty acids (carboxylic acids with a single long hydrophobic 'tail').

Fatty acid vesicles, membrane bilayers that enclose an aqueous volume, are a particularly attractive experimental model for protocells because they exhibit a remarkable ability to grow and divide (i.e., self-reproduce) without biological machinery. To grow by 'feeding', newly added amphiphile molecules must insert into an existing membrane more quickly than they self-assemble into new vesicles. Insertion of more than a trivial amount requires both association with the outer leaflet and flipping into the interior leaflet of the bilayer, to avoid an untenable imbalance between the leaflets. Flipping is also required for a competitive mode of growth, in which osmotically or chemically stressed vesicles 'steal' lipids from other vesicles. Flipping is quite slow in today's cell membranes, whose two-tailed phospholipids have flip-flop rates so low that enzymes (flipases) are needed. But fatty acids, having a small head group and a single tail, flip quickly. Fatty acids are unlikely to be unique in this respect, so studying different lipids and lipid mixtures is a promising area of current research. In addition, growth by vesicle fusion is quite general and is routinely used with phospholipid vesicles. Vesicle division can be achieved readily with physical forces, and even gentle shaking in a flask can be sufficient for vesicles with excess surface area to undergo fission. Vesicles have become prominent protocell models due to the richness of such behaviors.

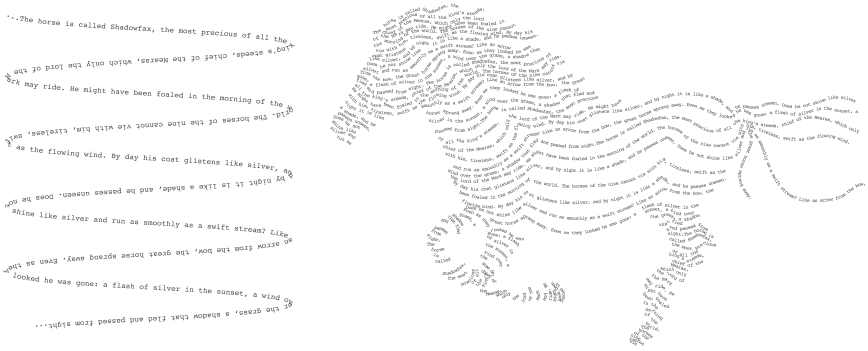

Figure 1. The dual nature of RNA.
The information string (left) also adopts a functional form (right). Text adapted from *The Lord of the Rings* (1954–55), by J.R.R. Tolkien. Image created by artist Lillian McKinney.

The practical implementation of protocells, such as ribozymes inside vesicles, immediately raises interesting challenges and lessons. For example, ribozymes usually require relatively high concentrations of divalent cations for optimal activity, but these ions also accelerate RNA degradation and destabilize fatty acid vesicles. Chemical compatibility is, therefore, an important issue, whose solution might require the addition of more components (e.g., chelating agents), thus increasing the complexity of the system. In addition, the chemical and biophysical environment inside a protocell differs from dilute aqueous solution. The membrane crowds macromolecules, promoting compaction; effective concentrations may be increased due to the attoliter volumes; and chemical interactions with the membrane may affect ribozymes and substrates in ways difficult to predict. While protocells bring these issues to the fore, the lessons learned about their emergent properties contribute to knowledge and intuition that may be more widely applicable.

In the field of synthetic biology, many cell-like systems of varying levels of complexity are being studied for a variety of purposes. All of these systems are much simpler than any extant living cell, and they include artificial cells, synthetic cells, minimal cells and protocells. In general, systems designed to partially mimic the function of a modern cell, such as for bioproduction of desired chemicals or investigation of specific biological phenomena, are called 'artificial' or 'synthetic cells'. They may contain, for example, a gene circuit with a transcription–translation extract. These bottom-up systems carry out specific functions and are usually not intended to self-reproduce. On the other hand, cells that are composed of a minimal set of genes and other components needed for life are usually called 'minimal cells'. These are made by a top-down approach of reducing the genome of an extant organism (usually highly heterotrophic, e.g., mycobacterium) as much as possible without impairing survival and reproduction in a given environment. Both artificial cells and minimal cells behave similarly to modern cells, by intent or descent. Protocells, on the other hand, need not be either structural or functional mimics of modern cells. Instead, they are experimental models for constructing a basic form of life, often inspired by ideas about the earliest cells on Earth, intended to inform the construction of an entity capable of open-ended evolution.

Alternative compartments

Vesicle membranes are not the only way to create compartments. Emulsions can also form readily

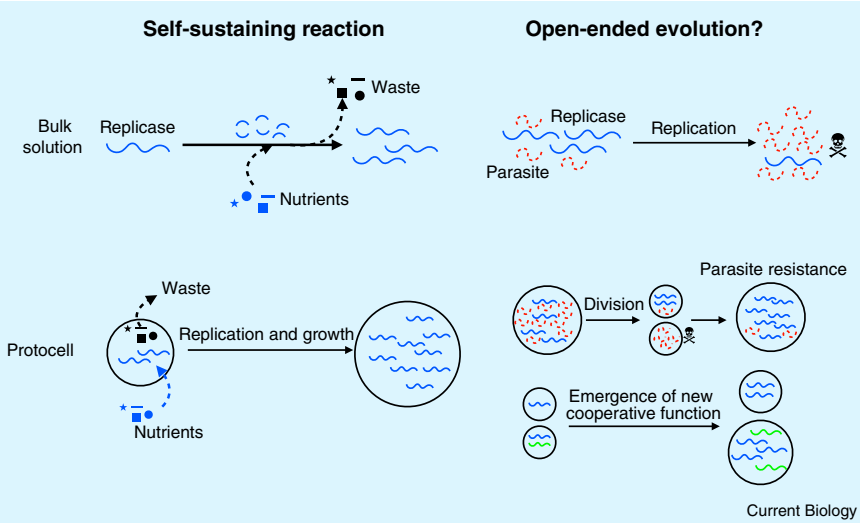

Figure 2. The importance of cells for life.
A replicase (blue; top left) takes in nutrients and outputs waste products during replication. Selection in free solution favors parasitic sequences (red), which lack activity but can be copied faster (top right), leading to collapse of the replication system. In the protocell, the growth, division, and internal replication are coupled (bottom left). A semi-permeable membrane is shown, allowing passage of small molecules. Physical compartmentalization, even if randomly segregated, prevents collapse and may enable open-ended evolution, such as the emergence of new cooperative functions (green).

(e.g., water-in-oil droplets formed by vigorous mixing of aqueous solution and oil in the presence of stabilizing surfactants), but these require a fair quantity of immiscible liquid. Although not really prebiotically plausible, emulsion-based studies are experimentally tractable and are particularly useful for evolutionary studies. A gratifying example is a compartmentalized version of the Spiegelman experiment carried out by the Yomo and Ichihashi groups; indeed, the compartments prevented parasites from overtaking the system. Another possibility, initially proposed by Oparin in 1938, is liquid–liquid phase separation (LLPS), which produces highly concentrated droplets with a composition distinct from the surrounding dilute aqueous solution. Complex coacervates are formed through LLPS of a solution of two oppositely charged polyelectrolytes that attract to form an electrolyte-dense phase. Hence, complex coacervates collect multiple RNA molecules into a small volume. LLPS has received quite a bit of attention in recent years as a mechanism for the formation of non-membrane-bound organelles, such as nucleoli and P granules, and their potential as a protocell model is currently an active area of research.

While all of these approaches (vesicles, emulsions, and LLPS) provide compartmentalization, their biophysical properties differ in fundamental ways. The inside of a vesicle and emulsion droplet are somewhat similar, both being mostly water bounded by a layer of surfactants. On the other hand, a coacervate is a highly condensed polymer assembly, somewhat akin to a loose pile of spaghetti. The concentration of monomer equivalents in this phase is on the order of 1 M, and water occupies only about two-thirds of the volume. For RNA, the coacervate droplets can be enriched roughly thousands-fold despite being dispersed in a polymer-deficient aqueous phase. In such an assembly, reaction rates, folding, and other properties can be quite perturbed.

Another important aspect to consider is permeability. Small molecules, such as nutrients and waste products, must be able to cross from the environment into the protocell. However, genes themselves (e.g., RNA) should not exchange, or should exchange only slowly, among protocells to preserve genetic integrity and avoid parasites. The three systems differ substantially in this regard. Water-in-oil emulsions are essentially impermeable to polar molecules due to the hydrophobic 'ocean' surrounding the droplets; little to no RNA is exchanged among emulsion droplets unless by fusion. By contrast, coacervates, having no hydrophobic barrier, exchange small molecules and even RNA oligonucleotides readily. Membrane bilayers lie somewhere in between, being permeable to small molecules, even mononucleotides (for fatty acids) but impermeable to larger RNAs. Membranes formed by amphiphiles with a single alkyl chain, such as fatty acids or related compounds, are more permeable than membranes made from double-chain lipids (which are the main components of the extant cell membrane).

What about self-reproduction of the compartments? As with vesicles, for emulsion and coacervate systems could grow via fusion. Indeed, emulsion and LLPS droplets represent kinetically trapped states. Left alone for long enough, droplets exchange material, leading to Ostwald ripening, and eventually coalesce into a bulk phase. Interestingly, for both droplets and vesicles, growth and division can be coupled. When 'fed', both types of structures can undergo a shape instability driven by the chemical input. This instability, much like the Rayleigh instability seen at dripping sink faucets, results in pearling and division. Otherwise, both droplet and vesicle 'division' can occur through physical agitation. The rates of these processes relative to replication and metabolism are an important consideration for the integration of the protocell.

A final factor to consider is the prospect for the system to develop open-ended evolution. Viewed through this lens, protocells with bilayer membranes have an advantage over membraneless compartments, since they are close analogs of a system that does evolve in an open-ended way (i.e., our own biology). Other forms of compartmentalization might, or might not, require additional transformations to attain open-ended evolution, such as through hybrid systems that

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combine desirable properties (e.g., coated droplets). At the same time, exploration of alternative compartment types probes the chemical limits of life and may well have unexpected relevance to our own biology.

Protocells at the intersection

Protocells occupy an interesting intersection of two intellectual approaches in biology. The spirit of bottom-up synthetic biology is famously captured by Richard Feynman's final blackboard writing "What I cannot create, I do not understand". The practical process of creating a protocell requires grappling with fundamental chemical and biophysical realities in pursuit of understanding the cell as a unit of life. On the other hand, systems biology is broadly concerned with interactions among parts of complex biological systems, to understand the 'whole' as more than the sum of its parts. Systems biology is usually applied to extant biology, i.e., thousands or millions of parts requiring high-throughput techniques and computational models with many parameters. For the protocell, even though there are many fewer parts, a deep level of mechanistic understanding of interactions among parts is integral to its practical construction, particularly for developing and recognizing emergent behaviors. The study of protocells thus merges ideas from systems biology and bottom-up synthetic biology.

The journey toward a chemical system capable of open-ended Darwinian evolution faces many challenges. Yet progress is evident in several directions. For example, while it is not yet clear how to couple the rates of various processes to achieve a self-sustaining life cycle, the discovery of several interesting mechanisms (e.g., the coupling of growth and division, the coupling of membrane stress and growth) suggests that this may be achievable with relatively few interacting components. Another area of interest is the first synergy between ribozymes with different functions, leading toward greater complexity. While natural selection could favor cooperating ribozymes through multilevel selection or other mechanisms, how exactly such a system would arise in practice represents an important subject for detailed study. Finally, the environment plays a pivotal role in natural selection. Which environment would present selection pressures on the protocell that would stimulate the emergence of new functions and greater complexity without destroying the system? Like the hydrogen atom in physical chemistry and E. coli in molecular biology, protocells are simple experimental models that serve as a focal point while moving toward a greater understanding of cells themselves.

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Primer
Fossil cells

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Although the fossil record may be commonly perceived as a trove of old bones and shells, for most of Earth's history — its first four billion years — it is comprised almost exclusively of cells, their cell walls or resting cyst stages. Even in more recent history, those old bones preserve a record of the cells that precipitated the mineralised tissues in which they are now petrified. Hence, fossils have the potential to provide unique insights into the evolution of cells — from the emergence of cellular life itself, through many of the major evolutionary transitions including eukaryogenesis, multicellularity, sexual reproduction and cellular differentiation, and even cellular insights into genome evolution through deep time.

Life in a cell

Divining the very origin of cellular life on Earth is a tricky business, even assuming that the last universal common ancestor of extant life (LUCA) was a cellular organism rather than an unappetisingly thin genetic soup. While there are truly ancient (4.1–3.8 billion years ago) geochemical records of enrichment of the light carbon isotope that life prefers, this could have resulted from abiotic processes. Conclusive evidence for the oldest cellular life requires either biomolecular fossils of cell membranes, isotopic evidence of metabolism that evolved after LUCA or fossil remains of the cells themselves.

The 3.46 billion year old Apex Chert microbiota of the Pilbara Craton, Western Australia holds perhaps the oldest coherent claim for life. Originally described as an unanticipatedly early diversity of microbial organisms including possible photoautotrophs, the Apex microbiota appeared to evidence an early emergence of LUCA and its subsequent diversification. Some of the fossil remains (Figure 1A,B)