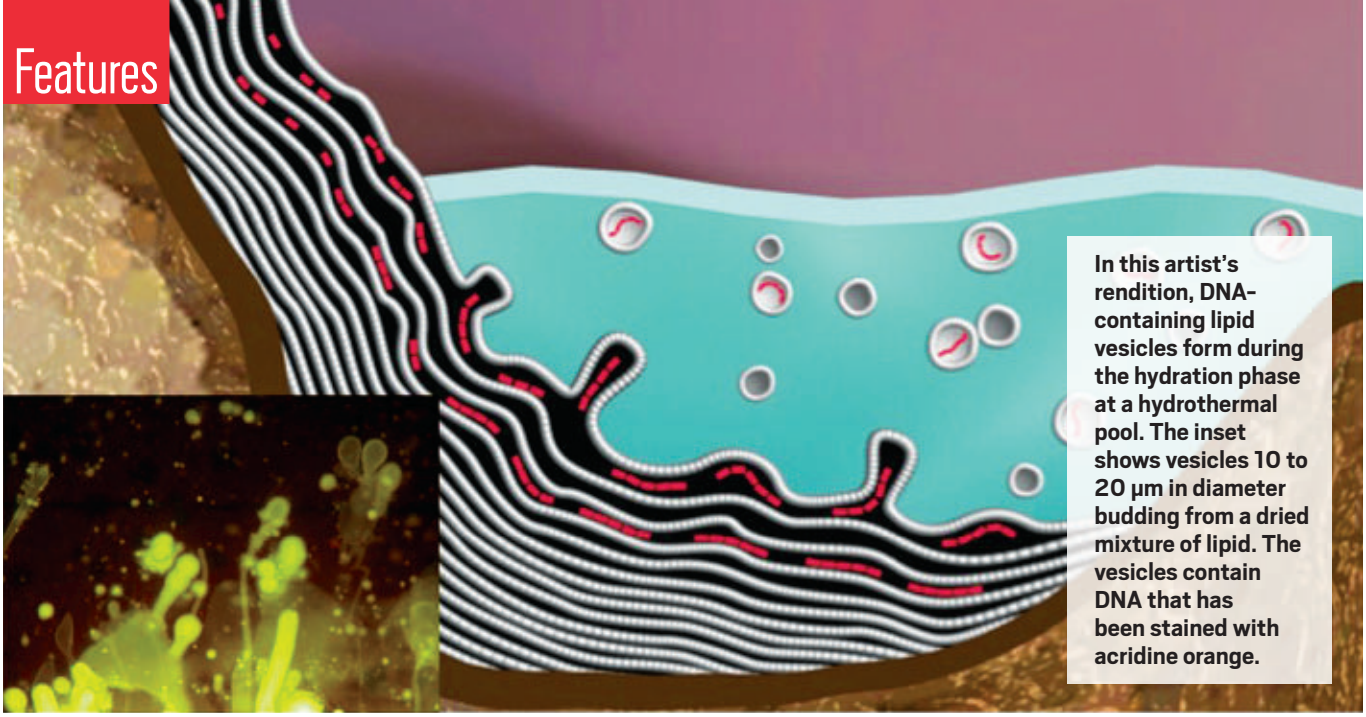

In this artist's rendition, DNA-containing lipid vesicles form during the hydration phase at a hydrothermal pool. The inset shows vesicles 10 to 20 µm in diameter budding from a dried mixture of lipid. The vesicles contain DNA that has been stained with acridine orange.

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ORIGINS OF LIFE

With protocells, scientists probe the chemistry that started biology

Researchers design cell-like compartments to figure out how Earth's first cells might have developed

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Unfortunately for scientists, there is no videotape of the universe's history that they can rewind to watch how life got started on Earth. Instead, they must recapitulate life in the lab to find plausible models of what happened billions of years ago. They start with chemical mixtures that might have formed under conditions on early Earth and see how those scenarios play out. They especially want to figure out how cells might have formed.

For example, scientists have made significant progress in showing that potential building blocks of life can spontaneously form from simple mixtures. But building blocks alone do not equal life. Scientists are now trying to figure out how those building blocks could assemble into something we might recognize as an early cell.

"There might be many conditions that give you suitable building blocks," says Wilhelm T. S. Huck, a researcher at Radboud University who is trying to construct living cells from simple compounds. "The key problem is how you get from those building blocks—whatever they were exactly—to something that would really be considered a living cell."

To try to solve that problem, scientists study so-called protocells. These cell-like compartments can take many forms: Most protocell researchers work with nucleic acids or other biomolecules enclosed in lipid vesicles, but some researchers think that protocells might not have needed membranes. Some teams have included modern polymerase enzymes in their compartments, but others want to find nucleic acids capable of catalyzing their own replication.

But none of these protocells have yet integrated the three capabilities that scientists equate to life: storage of information (including the instructions for self-replication), metabolism, and Darwinian evolution.

Instead, much of the protocell work has focused on the first capability, information storage. Multiple teams have recently shown ways that protocells can grow and divide, both of which are steps on a possible path to self-replication.

In one study, Tadashi Sugawara of Kanagawa University and coworkers described protocells that went through four steps to divide, demonstrating a primitive model of a conventional cell cycle. These protocells, which consist of bacteria-sized lipid vesicles, enclose DNA and polymerase enzymes to replicate DNA (*Nat. Commun.* 2015, DOI: 10.1038/ncomms9352). In addition to the phospholipids POPC and POPG, the vesicle membranes contain another cationic lipid and an amphipathic catalyst, which has an imidazolium head group and a lipid tail. The catalyst can form a complex with DNA and the cationic lipid that generates more of the lipid from precursor molecules.

During the first step of the cycle, the ingestion phase, an increase in the solution's pH causes the protocells to fuse with so-called conveyor vesicles that deliver nucleotides. In the replication phase, the protocell's DNA polymerase uses those nucleotides to make more DNA. In the maturation phase, replicated DNA protrudes into the membrane to form the catalyst complex. The researchers add lipid precursors, and this complex starts generating lipids. As a re-

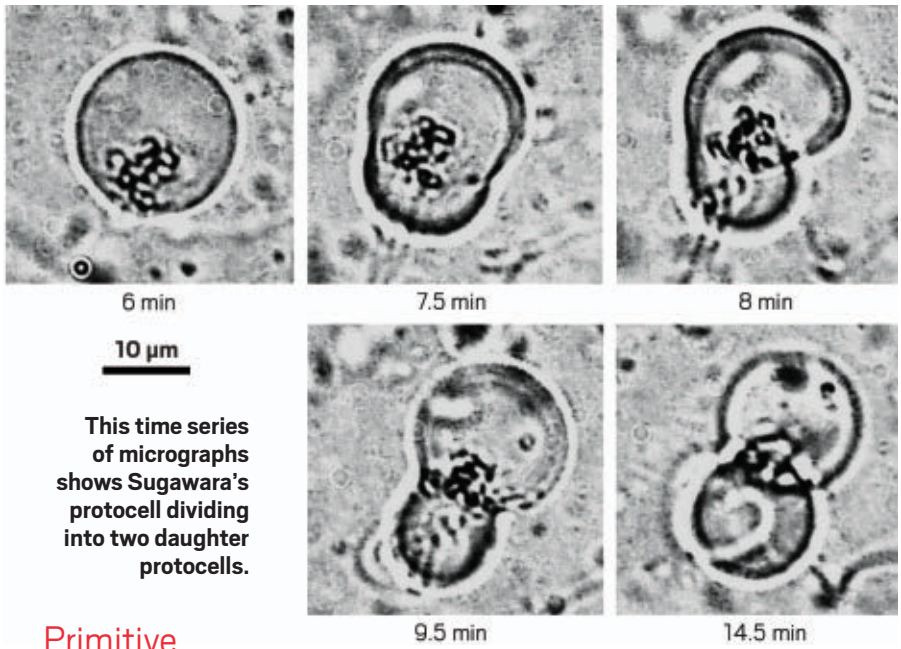
sult, the protocell membrane starts to grow. When the membrane becomes unstable, the protocell divides into two daughter vesicles during the final phase of the cycle.

The researchers demonstrated two complete cycles but claim more cycles should be possible. "Our model protocells continue to replicate DNA as long as nucleotides and the polymerase are occasionally supplied from outside," Sugawara says.

Vincent Noireaux, a biophysicist at the University of Minnesota, Twin Cities, who also develops protocells, notes that each step in Sugawara's model cell cycle is triggered by an external stimulus. "It's great work, but there is still a lot to accomplish and understand to get a fully autonomous compartment capable of sustaining itself without any sort of activation from outside." Still, he acknowledges that "devising a minimal metabolism capable of biosynthesis for self-reproduction is incredibly challenging."

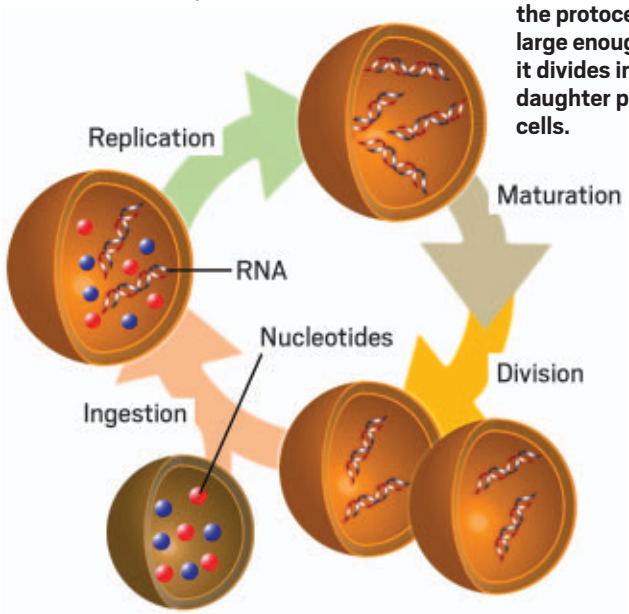
Another team, led by Tetsuya Yomo of Osaka University, has also reported vesicles that can reproduce over multiple generations (*Proc. Natl. Acad. Sci. USA* 2016, DOI: 10.1073/pnas.1516893113). They mix two liposome populations, one that contains RNA and another that contains nucleotides and the enzyme RNA replicase, which copies RNA. The researchers use freeze-thaw cycles to fuse the liposomes and stimulate RNA replication. When the liposomes reach a certain size, they divide into smaller liposomes. Yomo and coworkers were able to sustain the system for 10 cycles.

Both Yomo and Sugawara use membrane components found in modern cells instead of simpler compounds that may have been floating around on early Earth. That's because they're less interested in how life originally began on Earth than how life arises from chemistry.



This time series of micrographs shows Sugawara's protocell dividing into two daughter protocells.

Primitive model cell cycle



Sugawara's model cell cycle has four stages. In the ingestion phase, vesicles deliver nucleotides to RNA-containing protocells. In the replication phase, the protocells use those nucleotides to copy the RNA. In the maturation phase, lipids from the vesicles are incorporated into the protocell membrane. Finally, the protocell grows large enough that it divides into two daughter protocells.

"I am interested in constructing a molecular system that exhibits dynamic events, such as self-reproduction, recursive proliferation, and evolution, which must be an essential feature of life," Sugawara says. "We cannot address questions about the origin of each material, such as protein, RNA, and POPC, but we can address the question of how those materials mix to start targeted cellular functions," Yomo says. Because he uses modern biological molecules, Yomo avoids calling his structures protocells. "It's better to call it an artificial cell model," he says.

While Yomo, Sugawara, and most other protocell researchers study their systems inside laboratories, David Deamer and Bruce Damer of the University of California, Santa Cruz, have gone out on field trips, searching for conditions that approximate those on prebiotic Earth. Volcanic hydrothermal pools seem to fit the bill.

Deamer recently visited the Bumpass Hell section of Mount Lassen, a volcano in California. Here, sulfurous volcanic gases waft up from holes in the hydrothermal field called fumaroles. Such areas go through wet/dry cycles with rain or hot springs filling puddles that then later evaporate.

In his experiments, Deamer exposes a mixture of lipids and nucleotides for a couple of hours to the acidic gases coming out of the fumaroles. After the mixture dries out, he takes it back to the lab and extracts polymers from the lipid matrix. "We get something that looks like RNA," he says.

In previous work, Deamer and colleagues at McMaster University showed that the nucleotides in a lab version of this experiment are trapped between lipid layers (*PLOS One* 2013, DOI: 10.1371/journal.pone.0062810). It's only a small step from there to a protocell in which the polymerized nucleotides are encapsulated by a lipid membrane.

"I encourage my colleagues to take their experiments to a place like Bumpass Hell," Deamer says. "Most of them rebel against that idea because it seems so uncontrolled. But they're missing so much that can happen in these conditions."

Yet not all researchers venture to volcanic fields to try to mimic prebiotic conditions. For some of these lab-bound scientists, the goal is to replicate nucleic acids inside protocells without enzymes, because enzymes are not likely to

COURTESY OF TADASHI SUGAWARA (CELL CYCLE); *NAT. COMMUN.* (MICROGRAPHS)

have been part of primitive cells.

"You can't have evolution unless the genetic material is able to replicate," says Nobel Laureate in Physiology or Medicine Jack W. Szostak of Harvard Medical School, who studies protocells. But there's not yet a way of doing complete cycles of RNA replication and cell division without enzymes.

Szostak wants to figure out whether membranes might promote RNA reactions without modern enzymes. Such a process would require a way for RNA to get transported to the interior surface of a membrane. Last year, Szostak's group showed that amphipathic peptides can direct RNA molecules to vesicle membranes (*Angew. Chem. Int. Ed.* 2015, DOI: 10.1002/anie.201505742).

They developed a system involving tiny, 100-nm-sized vesicles encapsulating undecylimidazole—a model for cationic, amphipathic peptides—and larger, 5- to 25-μm-sized vesicles containing RNA. When the two vesicles were mixed, the tiny vesicles brought the undecylimidazole to the surface of the larger vesicles. Within a short period of time, the RNA started to associate with the membrane.

Researchers have focused on protocell replication because it's necessary for evolution, which most people consider to be a defining characteristic of biology. "Only a protocell that gained evolvability could have led to present cells," Yomo says.

But Kepa Ruiz-Mirazo, who studies origins of life at the University of the Basque Country, thinks metabolism is the hardest part of the protocell problem. Modern metabolism depends on enzymes. "But you can't start with enzymes. They are the result of the whole process," he says. If you look at the possible sources of prebiotic molecules, such as meteorites, there are no enzymes. "Enzymes are the product of life and probably came last in the origins of life," Ruiz-Mirazo says.

Ricard Solé, who heads the Complex Systems Lab at Pompeu Fabra University, thinks that modeling the thermodynamics underpinning protocells could point the way to a nonenzymatic metabolism. His group has computationally and theoretically modeled the physical chemistry of a system in which the lipid decanoic acid aggregates into a membranelike structure around decanoic anhydrides, which are precursors of the lipid molecules.

"It was an extremely simple metabolism, which was essentially a single reaction that transforms precursors in the soup into lipids," Solé says. "When the precursor molecules approach the membrane, they quite easily become new lipids, so the protocell keeps growing. But eventually this destabilizes and splits." With a steady supply of precursors, the model predicts that the system can undergo many cycles (2015, arXiv: 1503.04683v1). Solé describes it as the minimal self-replicating model that doesn't involve RNA or other information storage.

Still, a protocell with all three capabilities is a long way away. "I'm optimistic that somebody will get to a point where we have a functioning protocell model where the vesicle compartment replicates and its nucleic acid contents can both replicate and carry out simple functions," Szostak says. "That opens up a lot of interesting new questions we can ask." For example, researchers could then study how different chemical and physical conditions affect the evolution of the system.

"What we can do as scientists is show what is possible, that there are conditions that allow molecules to organize in a certain way," says Pasquale Stano, an origins-of-life researcher at Roma Tre University. "We're not going to prove that life originated in a certain way but that, given some conditions, we don't need a miracle." ■