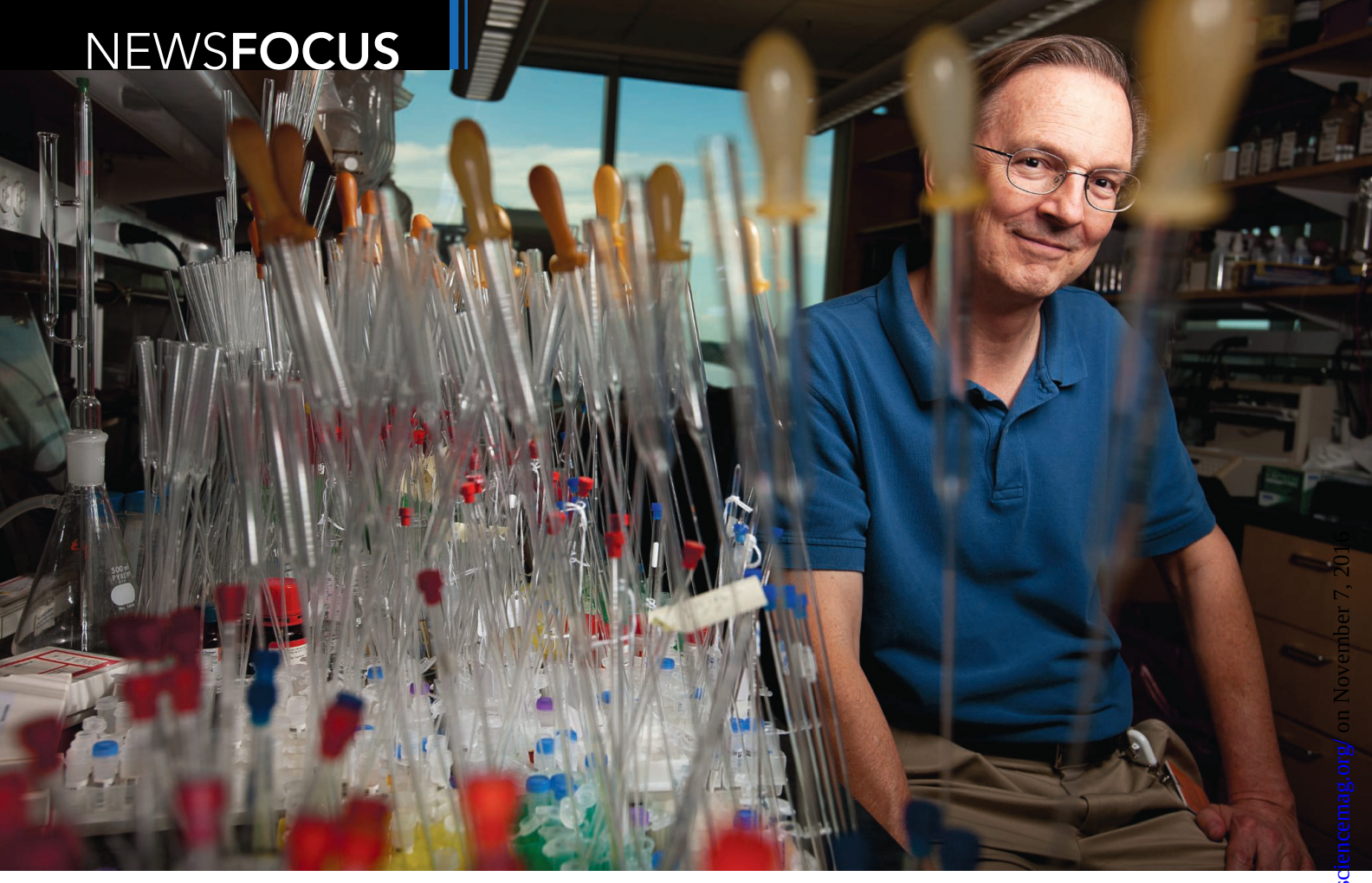

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The Life Force

Step by grueling step, Jack Szostak is pushing through the barriers that keep him from his goal: making living cells from scratch in the lab

Jack Szostak knows he'll never realize his ultimate scientific dream. But if he pulls off number two on his list, "it will go down in history as the greatest experimental achievement ever," says John Sutherland, an organic chemist at the Medical Research Council's Laboratory of Molecular Biology in Cambridge, U.K. Not bad for a backup.

Szostak, a molecular biologist at Harvard University and Massachusetts General Hospital in Boston, has already accomplished some spectacular science. He shared the 2009 Nobel Prize in physiology or medicine for helping to reveal the role of telomeres, the end bits of chromosomes that help protect genetic instructions during cell division. But more than a decade ago, Szostak shifted his lab's focus to exploring how life on Earth may have gotten its start. He would dearly love to know the recipe for the primordial soup in which it all began some 4 billion years ago. That recipe is almost assuredly lost to history. "We don't have a time machine," Szostak says. "We can't go back."

So he hopes to do the next best thing: fiddle around with a few ingredients of his own and watch as they spontaneously assemble themselves into genes inside simplified cells that copy themselves and demonstrate the first emergent signs of Darwinian evolution. The origin of life. Again. Only this time in a lab.

A lab demonstration wouldn't prove that life emerged the same way, Szostak says, but it would begin to tell a plausible story about how chemistry made the transition to biology. "If we can do that, to me it would give us a pretty good understanding of how life got started."

It's a big if. But on page 1098, Szostak and Katarzyna Adamala, his former graduate student and now a postdoctoral associate at the Massachusetts Institute of Technology in Cambridge, report taking a major step in that direction. For the first time, they found a recipe that promotes RNA copying inside primitive "protocells." It's not life in the lab—not yet—but other origin-of-life researchers are watching closely, says Gerald Joyce, a chemist and origin-of-life researcher at the Scripps Research Institute in San Diego, California. "You never want to bet against Jack," Joyce says. "He has a really good nose for where to go."

For an RNA-containing protocell to display Darwinian evolution, Szostak says eight large problems must be surmounted (see table, p. 1034). His lab has already solved three, and he says it is closing in on another three. That leaves two to go. "It's tantalizing," Szostak says. "We're close." And he's not the only one who thinks so. "I'd be hugely surprised if we don't get to that [during my career]," says Matthew Powner, a former postdoctoral assistant of Szostak's who now runs his own lab at University College London. "There is tangible excitement that this can be solved and this will mean something big."

In the beginning

In tackling the origin of life, Szostak is taking on one of the biggest questions humanity has ever asked—second only to the origin of the universe itself. For millennia, it lay in the realm of philosophy, theology, and alchemy. Science got in on the act in a systematic way in the mid-20th century, after researchers discovered the structures of DNA and RNA and

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Creation scientist. Jack Szostak is working to recreate a recipe that transformed chemistry into biology.

their central role in coding for proteins, the chemical workhorses of the cell. In a host of now-classic experiments, scientists probed how potential building blocks of life such as amino acids and nucleic acids could be synthesized from simple compounds under conditions thought to have prevailed on early Earth. Progress was rapid and spirits high. "Laboratories will be creating a living cell within ten years," Colin Pittendrigh, an American biologist, predicted in 1967.

Then things got complicated. Researchers realized that creating the raw ingredients of life wasn't enough: They also needed to explain how those compounds assembled themselves and evolved into the sophisticated living cells on Earth today. Life required not just the right ingredients, but also the right molecular tools. In the late 1960s, a trio of biologists—Francis Crick, Carl Woese, and Leslie Orgel—independently proposed that RNA could serve two roles. What came to be known as the "RNA World" hypothesis holds that RNA existed long before DNA, catalyzed its own reproduction, and helped give life its start. Others believed RNA wasn't up to the task and proposed alternatives for the earliest biochemistry, developing the "peptide world," "lipid world," and "metabolism first" scenarios for life's origin. Conferences on the subject became shouting matches. "They all fought each other tooth and nail," Sutherland says. "People wondered, 'How on Earth do you solve this problem?' "

Throughout most of history, the answer had been simple: divine intervention. Szostak, though, takes pleasure in pushing back the borders of the supernatural. "To me it's very satisfying to find natural explanations for problems that were so complex that people had to resort to magic," he says. But he insists that he is not a philosopher; he simply likes to solve problems at the lab bench.

Szostak has had a practical bent for most of his life. The eldest child of an aeronautical engineer father and a mother who held down various jobs, he grew up in Ottawa and Montreal. As a child, his parents took him to church and Sunday school but weren't particularly devout themselves. "When I was 12, I said I'm not going to do that anymore," Szostak says; his parents seemed more relieved than anything else.

In his teens, Szostak became absorbed in chemistry. His mother was working as a librarian for a chemical company and used to bring home ingredients for his basement lab. His early experiments left "a few little scars," Szostak says. But he chuckles, "I still have all my fingers."

After earning an undergraduate degree at McGill University in Montreal in 1972, Szostak moved to Cornell University to work with biologist Ray Wu. Wu's lab was racing to synthesize DNA fragments that could detect messenger RNA—the form of RNA that carries copies of genes to ribosomes, which translate their code into proteins. Wu's lab lost out by a few months to British biologist Michael Smith. Szostak didn't come in second often after that.

> **"The big picture is it's not an RNA world, a peptide world, a lipid world. It only works if everything is connected."**
>
> —JOHN SUTHERLAND, MRC LABORATORY OF MOLECULAR BIOLOGY

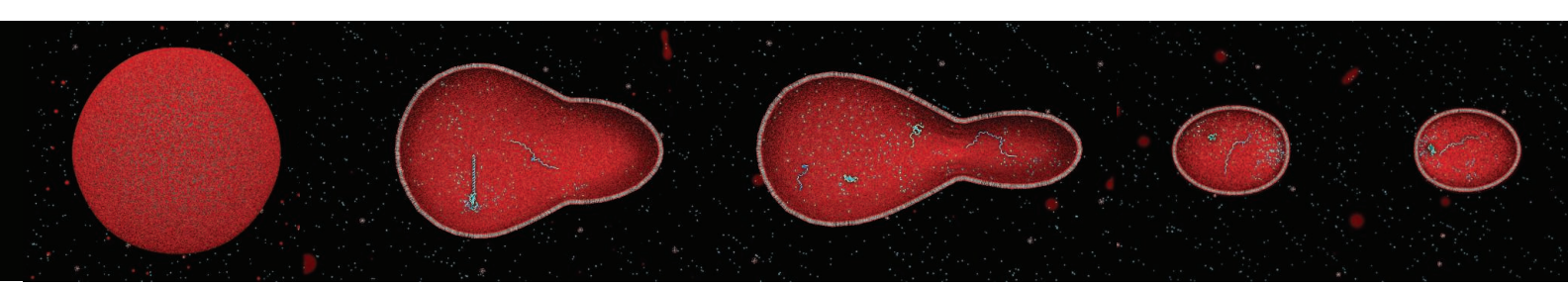
After setting up his own lab, Szostak plunged into the burgeoning field of genetics. He helped develop the yeast artificial chromosome, a technique that was widely used to identify, clone, and manipulate genes. He identified the specialized sequences of telomeres and helped show how they aid in cell division and how telomeres contribute to cell aging, hereditary diseases, and cancer.

Szostak's success brought other researchers flocking to work with telomeres. "The field was getting crowded," Szostak says. "I thought maybe it was time to do something different." He drew inspiration from experiments by Thomas Cech of the University of Colorado, Boulder, and Sidney Altman of Yale University, for which they won their own Nobel in 1989. In the early 1980s, Cech and Altman found that RNA not only serves as a genetic mail carrier but can also catalyze chemical reactions. Because that role was previously thought to be the sole domain of proteins, the finding bolstered the RNA World hypothesis.

In the early 1990s, Szostak switched his lab's focus to RNA catalysts, known as ribozymes. He and his colleagues invented a scheme for evolving new ribozymes in the lab, in a process known as in vitro selection. (Joyce's group at Scripps carried out similar work.) In 1995, Szostak and former students Eric Ekland and David Bartel used the technique to produce the first RNA catalyst capable of welding two other pieces of RNA together. A year later, Ekland and Bartel announced that they had found an RNA catalyst capable of serving as an RNA polymerase, the enzyme that living cells use to produce new copies of an RNA strand.

RNA was proving increasingly versatile, with multiple roles previously reserved for DNA and proteins. In 2000, researchers at Yale discovered that even the catalytic heart of the ribosome is an RNA-based ribozyme. Here was a possible relic of the RNA World, strongly supporting the idea that early life ran on RNA and only later evolved the ability to build chemically superior proteins.

Szostak found himself thinking more and more about the RNA World. The hypothesis had its problems, he realized. "RNA brings with it a lot of baggage," Szostak says. It is a fragile molecule, so researchers would need to explain how it could have survived conditions on early Earth. They would also need to explain how long RNA chains formed, were copied, split apart, and sent to daughter cells—the cycle of replication that is basic to life.



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Divide and conquer. Protocells that assemble themselves and split might undergo Darwinian evolution.

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Most fundamentally, it wasn't at all clear how an RNA fragment drifting around in a warm pond or stuck on a fleck of mineral could have spawned variants that would have reproduced more or less rapidly, allowing "fitter" variants to outcompete others. What allowed primordial RNA to *evolve*?

All of the above

After numerous conversations with other origin-of-life researchers, Szostak became convinced that RNA couldn't have done it alone. The molecules needed to be isolated and confined. Some sort of cell membrane probably was needed, both to concentrate the ingredients of life and to promote a Darwinian process. "If [chemistry] is compartmentalized, you keep molecules related by descent together," Szostak explains. If an RNA-containing protocell arises and can grow and divide better than its neighbors can, it can pass its advantages to its progeny. The protocells would allow fitter molecules to flourish, in true Darwinian fashion.

"I thought, 'Well, I've never worked on membranes before,' " Szostak says. " 'Maybe it's time to do so.' " Protocell membranes, he knew, must have been very different from those of modern cells. Current cell membranes are made from fats called phospholipids and are all but impenetrable to key ingredients of life such as amino acids and nucleic acids. Without the modern biochemical apparatus of protein-based pores and pumps, nutrients cannot get in and waste products can't get out.

Szostak and his students found an alternative. They discovered that far simpler fatty acid molecules could form leaky cell-like spheres that allowed ions, amino acids, and nucleic acids to diffuse in. In 2008, Szostak's team reported that RNA nucleotides, or building blocks, could enter these cells and then form growing RNA chains that were too big to diffuse back out. A year later, Szostak and his graduate student Ting Zhu found that adding extra fatty acid molecules to the mix caused existing protocells to grow. Then, modest shear forces—such as those that protocells might experience when flowing through a column of warm water near a volcanic vent—would stress the large spheres until they divided, and any RNA inside them would be partitioned among the daughter cells. Yet another paper showed that RNA or peptide catalysts would speed the incorporation of additional fatty acid molecules into protocells, promoting their growth. Crude as they were, fatty acid vesicles appeared to be up to the job.

What about the other key component, RNA? Advances both in Szostak's lab and elsewhere showed that, with the right mix of ingredients, individual RNA nucleotides would bind to a sister "template" strand in a copying process without the enzymes required inside modern cells. That was good news—but researchers couldn't make it happen inside a protocell.

The biggest problem was that one of the most important ingredients for copying an RNA template without added enzymes is charged magnesium ions (Mg^{2+}). Take away Mg^{2+} and the reaction proceeds so slowly, it's hard to imagine how it could have been relevant to early life. But Mg^{2+} has downsides. The ions rip apart fatty acid protocells and shred growing RNA chains as fast as they build them up.

Adamala says she tried adding hundreds of different compounds and short peptides to the mix. "Nothing worked," she says. "It was very frustrating." But then she turned to metal-binding compounds called chelators, and one gave her the result she was looking for. In their current paper, Adamala and Szostak report that when they added a bit of a simple citric acid derivative called citrate to the mix, they got a perfect Goldilocks result. The citrate bound the Mg^{2+} ions tightly enough to keep the ions from tearing apart either the RNA or the fatty acid membranes, but loosely enough to give the Mg^{2+} ions leeway to copy a template RNA strand.

"It's a beautiful paper," Sutherland says. Citrate itself is a tantalizing solution, he says. It also plays a key metabolic role in modern cells, which suggests that it, too, could be a molecular fossil left over from early evolution.

Equally important, Sutherland says, is that for the first time, all the various pieces of the protolife puzzle seem to be coming together. "The big picture is it's not an RNA world, a peptide world, a lipid world. It only works if everything is connected," Sutherland says. George Cody, an organic geochemist at the Carnegie Institution for Science in Washington, D.C., agrees. "In the beginning, all these had to be in play," he says.

Next, Szostak says, his team must overcome two large hurdles: The researchers must show how individual RNA bases could have become chemically "activated" so they would readily bind to growing RNA strands. Then they must demonstrate how RNA strands can duplicate without a starter template strand to help the nucleotides come together to form the complementary strand. Sutherland thinks these are solvable problems. "There's no reason it shouldn't be possible to recreate [a replicating cell]," he says.

Even if Szostak's experiment works, there will still be plenty of unanswered questions. Among them: What prebiotic processes would have produced the RNA nucleotides and other mix of ingredients that would have gone into an early protocell? It's also not clear that an evolving protocell made in the lab would have any broader significance, says Ramanarayanan Krishnamurthy, an organic chemist at Scripps. "Pushing its relevance to what happened 4 billion years ago is a risky thing."

But Szostak argues that such dismissals are too facile. Such a "cell" would help define the chemistry that must have been involved at some level to get a self-replicating system going. Sutherland likens it to a crossword puzzle. As you begin to fill in words in some of the open squares, the options narrow for the words that intersect each known word. The puzzle shrinks, making subsequent answers easier. For someone aiming to show that the puzzle of life's origin didn't solve itself by magic, that would be a satisfying result indeed.

–ROBERT F. SERVICE

Steps Toward an Evolving RNA Protocell

Challenge	Status
1. Enable RNA template copying to proceed despite strands with random backbone linkages	✓
2. Enable paired RNA strands to separate without high temperatures	✓
3. Keep metal ions (needed to copy RNA) from destroying protocells and RNA strands	✓
4. Improve accuracy of copying RNA without enzymes	Recent progress
5. Speed up rate of copying RNA without enzymes	Recent progress
6. Keep RNA strands from quickly reforming duplexes after they separate	Recent progress
7. Chemically "activate" RNA nucleotides to bond to a growing strand	Not demonstrated
8. Enable RNA to form in protocells without primer template strands	Not demonstrated

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Editor's Summary

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