

## What Came Before DNA?

**Scientists may soon reveal how life began on Earth—by creating a primordial organism in the lab**

By Carl Zimmer

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The question took me by surprise. I was sitting in a noisy Boston café with two biochemists who were having a straight-faced conversation about putting together a budget to create synthetic life-forms. Next to me was Jack Szostak of Harvard Medical School, and across the table was Steven Benner, who had flown up from the University of Florida to pay Szostak a visit. The conversation was thrumming along, touching on the efficiencies of chemical reactions and the like, when Benner abruptly turned to me and asked, “How much do you think it would cost to create a self-replicating organism capable of Darwinian evolution?”

The question was not “Will we ever create life?” but simply how much money creating life would cost. “Twenty million dollars,” I said, choosing the number completely at random.

Benner nodded. “That’s what Jack says.”

Szostak, whose large glasses and round face make him look like an affable owl, had been letting Benner do most of the talking. Now he smiled, nodded with a slow blink, and said, “Sounds right.”

*Sounds right?* As we strolled back to Szostak's lab, past the long lines of idling ambulances parked by the Massachusetts General Hospital emergency room, I did some calculations in my head. Sequencing the human genome cost roughly \$500 million, and essentially all that scientists had to show for the money was a long string of letters that make up human DNA. By contrast, for less money than a middling movie makes in a weekend, Szostak hopes to transform chemicals into a single-celled organism that will grow, divide, and evolve—and soon. “I think it's conceivable it could be done in as little as three years,” he said. “The number of steps that might be real potential roadblocks has declined almost to zero.”

What's more, Szostak's goal is not just to create life from scratch. His ultimate objective is bigger: Find out how life began on Earth. The fossil record and modern genetic analysis suggest that humans and all other living species are descended from bacteria-like microbes that first appeared about 4 billion years ago. But bacteria, appearances notwithstanding, are very complex. They can be packed with thousands of genes, along with proteins and other molecules, working together in an intricate struggle to stay alive. Most scientists agree that such DNA-based life probably emerged from a much simpler life-form that no longer exists on Earth. Szostak wants to figure out what that first life-form was by building it (or something close to it) in his lab.

Szostak, 51, embarked on this quest to re-create the ancestor of us all because he was bored with yeast. After years of studying yeast genes in search of insights into how human DNA works, he was looking for a challenge. He found it two decades ago after a spectacular discovery upended conventional wisdom about ribonucleic acid, or RNA, one of the fundamental building blocks of life.

Biochemists once viewed RNA as a lowly cellular messenger. Genes, made of double-stranded DNA, contain information for making proteins. This genetic code is embodied in long strings of chemical compounds called nucleotides and is copied onto RNA molecules, which then get shipped to ribosomes, biochemical factories where protein molecules are manufactured. Once completed, proteins curl up into complex shapes that let them do the actual work of life. Some proteins give an organism's body its structure, whether in the cell's internal skeleton or in a strand of hair. Other proteins, known as enzymes, can grab other proteins, cut them apart, or weld them to other proteins. DNA depends on enzymes to make new copies of its code as well as to translate it into RNA.

In the early 1980s Tom Cech, then a young biologist at the University of Colorado at Boulder, uncovered evidence that RNA does more than simply relay messages from DNA to proteins. In an experiment that earned him a Nobel Prize, he found that a single-celled creature named *Tetrahymena* possessed some RNA molecules that could act like simple enzymes. These molecules, which came to be known as ribozymes, twisted into a complicated snarl that allowed them to hack themselves apart. In other words, RNA could carry information like DNA and carry out biochemistry the way proteins do.



Courtesy of European Southern Observatory

#### MOLECULAR SEEDS

The key chemical precursors of life are abundant throughout the universe in interstellar dust and molecular clouds like those found in the Chamaeleon I constellation (above). Scientists imagine the embryonic Earth forming from such clouds in the early days of our solar system, roughly 4.5 billion years ago. In the primordial welter of planetary formation, the fundamental chemistry of life begins with formaldehyde, water, hydrogen cyanide, and ammonia. During a period spanning millions of years, as the nascent Earth coalesces and solidifies from the dust, these molecules are brought together and concentrated at the planet's surface. Comets and meteorites, which regularly shower the Earth prior to the formation of an atmosphere, scatter even more molecular seeds around the globe.

The discovery of ribozymes not only changed our understanding of how life works today, but it also offered insights into the origin of life itself. Scientists believe that life on Earth emerged from carbon compounds and other simple chemicals. But it has long been a mystery how those raw materials were transformed into DNA. After all, DNA can't survive without proteins. So the question has been: What came before DNA?

RNA could be the answer. Watching ribozymes at work revealed how primordial RNA could store genetic information and act like an enzyme. In theory, simple RNA-based life-forms could have spread and evolved for millions of years. Perhaps they eventually evolved the ability to assemble proteins as well as build DNA molecules. Because DNA and proteins did their jobs better than RNA, maybe they eventually took over these tasks.

Szostak saw in this theory a calling. "I thought, I can figure out something different to do, where we could contribute something," he says. In a world before DNA, RNA molecules would have had to be a lot more accomplished than the Tetrahymena ribozyme. Most important of all, RNA would have to function as an enzyme (known as a replicase) that could replicate other RNA molecules. So Szostak began to tinker with RNA molecules from Tetrahymena and other organisms to see if he could make one.

In 1991 he and graduate students Jennifer Doudna and Rachel Green succeeded in making a crude prototype. They created a molecule that could grab shorter chunks of RNA and make copies of them. It was a remarkable achievement, but Szostak knew it was only a small step toward something that could accurately be called alive.

Enzymes in living cells can make duplicate RNA sequences one nucleotide at a time. Szostak's ribozyme could only piece together chains of RNA, each of which was several nucleotides long. And his new molecule was grievously sloppy, making regular copying errors. In a single generation, it could turn a life-sustaining genetic code into sheer gibberish. To create a better molecule, Szostak decided to turn to the father of evolutionary theory, Charles Darwin, for inspiration: "We realized that if we were really going to have a chance to have an RNA replicase, we were going to have to evolve it."

For many years biologists have been able to witness evolutionary change in the laboratory by studying organisms such as fruit flies or bacteria. Using that research as a guide, Szostak and his students began building a system to allow RNA molecules to evolve as well. Evolution produces new adaptations through cycles of mutation and natural selection. Szostak started an evolutionary cycle by randomly stringing together nucleotides to create trillions of RNA molecules. Then he and his students gave the molecules a very basic task to perform: latching onto another molecule. Typically, only a few of these first-generation RNAs could do the job—and needed a long time to fumble around until they could grab the molecule. Szostak's team extracted the winners and made trillions of new copies, allowing some random mutations to creep in along the way. Then they set the new generation on the same task and picked out the ones that did the job fastest.

In each experiment, Szostak and his students repeated the process dozens of times. In the end they were left with RNAs that were exquisitely well adapted to the job at hand. Szostak named these evolved RNAs aptamers, which means "parts that fit." And fit they did. Aptamers turned out to be capable of performing an extraordinary range of tasks. Some aptamers can bind to a specific virus, and others can grab certain kinds of cells or attach themselves to vitamins.

Aptamers were just the beginning. Unlike aptamers, which are capable only of sticking to something else, ribozymes can change the structure of other molecules. So Szostak then adapted the same process to evolving specialized ribozymes. Some can cut DNA apart, and others can put it back together. But of all the



Photograph by Andrew Hetherington

#### WOULD-BE CREATOR

"Our ultimate goal is to create life," says biochemist Jack Szostak, sitting in his Harvard Medical School laboratory in Boston. Szostak's unassuming manner and modest work environment belie the audacious nature of his quest. Most of his research is conducted using conventional methods of molecular biology and relatively old-fashioned laboratory equipment.

ribozymes that now exist, the ones that fascinate Szostak most are the ones that can do what his handmade RNAs couldn't do: make new RNA.

#### SUGAR FIX

Aided by borate, the core molecules from the cosmos react to form ribose, a five-carbon sugar that is a key ingredient of the genetic material ribonucleic acid (RNA). These molecules may also combine chemically to make four organic molecules known as adenine (A), cytosine (C), guanine (G), and uracil (U). These organic molecules, called nucleobases, make the genetic "alphabet" of the RNA. Long sequences of the four letters spell out the essential biological blueprint of every living organism. Szostak and many other scientists believe that self-replicating RNA, or something similar, may have served as the earliest genetic material and that DNA—the primary genetic material in all living things on Earth today—appeared only after millions of years of evolution.

The best thing out there, says Szostak, is a molecule that had its origins in his laboratory. In 1993 David Bartel, then a graduate student with Szostak, produced a ribozyme that could join another piece of RNA to itself. In subsequent work at the Whitehead Institute and MIT, Bartel modified this type of ribozyme through tinkering and evolution. By 2001 he and his coworkers had something much closer to a full-blown replicase. The ribozyme could grab an RNA molecule that would act as a template. It would then use the template as a guide for adding nucleotides one at a time onto an RNA fragment. In total, the ribozyme could add on 14 nucleotides, with an accuracy of roughly 97 percent.

Today both Bartel and Szostak keep students and postdocs busy in their labs evolving improved ribozymes that can build longer copies. "What you really want is something that can go to 100 or 200 nucleotides and go completely every time," says Szostak. That's a big jump from what's available today and perhaps the biggest one still left before anyone can claim to synthesize life. But it's not much bigger than what has already been accomplished. "It's getting really close," says Szostak. "We don't have to worry about whether it's possible. We know it exists. Now we ask how we can tweak it to make it better or simpler."

Szostak's work with synthetic aptamers and ribozymes has convinced him that RNA could have once dominated the world. Meanwhile, other researchers have found evidence supporting the hypothesis in living cells. It turns out that RNA is far more versatile than scientists once thought. Last March, for example, biochemist Ronald Breaker at Yale University and his colleagues discovered that some RNAs self-destruct before they can be copied into a

protein if they grab onto a certain molecule. Other RNAs, he found, work the other way: Only if they grab a certain molecule can they act as a template for a protein. These "riboswitches," as Breaker calls them, are apparently essential to the workings of the cell. "The roles of RNA in the cell have expanded beyond what anyone imagined," says Szostak. "Who knows what else is lurking in there?"

The best proof that life got its start as an RNA-based organism would be to create one. But for all the advances to date, there's still plenty of work left to do before such a creature comes to life. A handful of ribozymes in a beaker—no matter how accomplished they may be—simply doesn't make the cut. It's as if Szostak wanted to prove that a car can exist; at this point, he's got brake pads, a steering wheel, and a lot of other parts strewn across a yard. Now he's got to get the pieces to work together.

The simplest way is to put the pieces in a container. All organisms alive today keep their DNA, RNA, and proteins together inside cell membranes. These oily bubbles prevent big molecules from getting out while letting smaller food molecules in. Today's membranes are complex constructions, built by a carefully choreographed crew of enzymes. Their surfaces are studded with sophisticated channels that carefully regulate what goes in and out of the cell. And as the cell grows, the enzymes expand the membrane as well; when the cell divides, enzymes push apart the membrane and its contents into two new cells.

All this takes lots of genetic guidance. A simple organism with only a sliver of RNA couldn't possibly build such a complicated container for itself. So four years ago, Szostak decided to expand his research on the RNA world: He set out to find a simple way to enclose his ribozymes.

DO OTHER CORNERS OF THE UNIVERSE HARBOR LIFE-FORMS WITHOUT DNA?

Under the appropriate conditions, phosphate reacts with ribose and nucleobases to form a large organic molecule called a nucleotide. Each nucleotide comprises one ribose molecule, one phosphate molecule, and one of the four nucleobases. Long strands of these nucleotides will ultimately link together to form RNA molecules. Phosphate and ribose act as the structural backbone holding the strands of nucleotides together. On modern Earth, volcanic exhalations and lava flows, such as those found at the Chain of Craters at Volcanoes National Park in Hawaii, produce rich concentrations of phosphate. The adolescent Earth was marked by periods of intense volcanic activity. Some scientists think the phosphate needed to make RNA most likely came from these frequent eruptions as well as from meteorites and existing mineral deposits.

The hunt for extraterrestrial life is heating up. In a decade, NASA hopes to launch a network of space-based telescopes that will be able to pinpoint Earth-like planets in other solar systems and see whether life has altered their atmosphere in the same way it has here on Earth—flooding it with oxygen, for example. Closer to home, scientists are designing devices that could be deployed on Mars or Europa, one of Jupiter’s moons, to detect microbial organisms. But it’s possible that even if extraterrestrial life exists, none of these searches will find it. That’s because everyone is trying to look for life in space that is like life on Earth. If life did emerge independently on some other planet or moon, it might be radically different, down to its very molecular basis.

All living organisms on Earth use DNA, but there may be other molecules that can do the job as well. For several years now, scientists have been learning how to attach the four nucleotides of our genetic alphabet to new backbones. Two kinds of artificial molecules—known as PNA and TNA—have proven particularly promising. Astrobiologists are paying a lot of attention to Harvard biochemist Jack Szostak’s efforts to produce a self-replicating RNA molecule. If Szostak and his colleagues succeed, they will have created the first self-sustaining life that does not depend on DNA. Of course, RNA is not profoundly different from DNA (it’s a single-stranded variation). But a self-replicating RNA molecule would open the door to new ways of thinking about life elsewhere in the universe.

—C. Z.

Two new members of his lab, Martin Hanczyc and Shelley Fujikawa, were willing to take on the challenge. They began by experimenting with fatty acids. These molecules, which make up the bulk of cell membranes, were likely to have been floating in the prebiological oceans of Earth. A number of nonbiological reactions can give rise to fatty acids; they’ve even been found in meteorites. Fatty acids also have the fortunate habit of being naturally attracted to one another, forming sheets that eventually curl in on themselves and create bubbles.

Hanczyc and Fujikawa began studying the bubbles, known as vesicles, to see if they could grow and divide like cell membranes without the help of a lot of cellular machinery. In the 1990s Italian chemist Pier Luigi Luisi figured out how to make vesicles grow by adding loose fatty acids to their solution; gradually, some of the molecules slipped into the vesicles and expanded them. Hanczyc and Fujikawa spent three years perfecting the process to make it more efficient. “Right now, 90 percent of the material we add gets incorporated into the vesicles we already have,” says Hanczyc.

Once Szostak's team proved that vesicles can grow, the challenge was getting them to divide. The researchers discovered a simple solution. They poured a solution of vesicles into a syringe and then squeezed it through high-tech polycarbonate filters. As the vesicles were forced through the 100-nanometer-wide pores, many of them were stretched out and pinched off to form into smaller vesicles, thanks to the natural attraction of fatty acids to each other.

#### ESSENTIAL INGREDIENTS

"Water is necessary for life," says Steven Benner. "At some point the nucleotide components had to move into an aqueous environment." Also essential are fats, from which cell membranes are constructed. In every organism, genetic material is housed inside a membrane that keeps dangerous substances out while letting in food and other necessary molecules. After the ribose, nucleobases, and phosphate combine to form nucleotides, fats are required to make this membrane. "Now that you've got your nucleotides, you have to capture them and put them into a cell," says Benner. Fat-forming molecules are potentially found throughout the universe in interstellar dust clouds, comets, and meteors. Fatty acids also form in Earth's oceans around hydrothermal vents. Some scientists believe that the earliest organisms emerged from these vents.

Without any help from enzymes, vesicles could grow and divide and grow again. And they did so under laboratory conditions that more or less mimicked some of the conditions on early Earth. Instead of squeezing through polycarbonate filters, for example, primordial vesicle-laden water might have squeezed through the pores of rocks around hydrothermal vents.

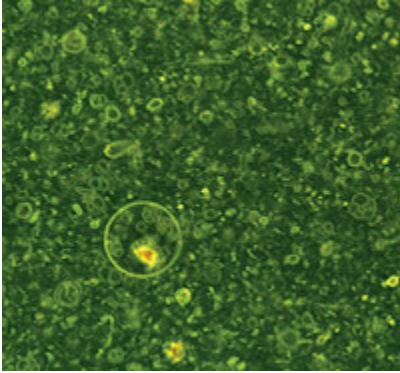
One afternoon in the summer of 2002, Szostak was sitting in his office when Hanczyc and Fujikawa walked in with a vial of murky liquid. His students had added a kind of clay known as montmorillonite to their solution of fatty acids. Somehow the clay sped up the rate of vesicle formation 100-fold. "We spent years working on getting the growth and division stuff to work. That was a pain," says Hanczyc. "But the clay worked the first time."

Clay had already proved to be potentially important in the origin of life. In the 1990s biochemist James Ferris of Rensselaer Polytechnic Institute showed that montmorillonite can help create RNA. When he poured nucleotides onto the surface of the clay, the montmorillonite grabbed the compounds, and neighboring nucleotides fused together. Over time, as many as 50 nucleotides joined together spontaneously into a single RNA molecule. The RNA world might have been born in clay, Ferris argued, perhaps the clay that coated the ocean floor around hydrothermal vents.

"The thing that's interesting is that there's this one mineral that can get RNA precursors to assemble into RNA and membrane precursors to assemble into membranes," says Szostak. "I think that's really remarkable."

As Hanczyc and Fujikawa analyzed their new vesicles, they made an even more remarkable discovery. Some of the grains of montmorillonite actually wound up inside the vesicles. Their next step was obvious. "It was very straightforward," says Hanczyc. "You just mix the RNA with clay, and mix it with the fatty acids, and voilà, you have RNA on the clay particles inside the vesicles."

Here was one possible way in which the pieces of the RNA world might have come together in cells that could grow and divide. The researchers haven't actually created synthetic life, but they may be within striking distance. "We have a pretty clear picture of what we have to do, and none of those steps look impossible," says Szostak.



Courtesy of Martin Hanczyc

### CLAYMATION CELLS

For laboratory scientists trying to re-create the primordial ancestor of all life on Earth, the challenge is to combine nucleotides, fatty acids, and water in an actual cell. This is a two-step process. Nucleotides must link together to form RNA strands, and then the RNA must be housed inside a fatty-acid membrane. Szostak and his colleagues were surprised to discover that a ubiquitous clay known as montmorillonite accomplishes both these tasks. When added to the molecular mix, the montmorillonite causes the nucleotides to spontaneously assemble themselves into RNA, which is then trapped inside fatty-acid bubbles called vesicles. The result is something that resembles a cell: It has genetic material and water contained within a waterproof fatty-acid pouch that, under the proper circumstances, could grow and even divide. An image of these makeshift cells (above), taken with an optical microscope and enhanced using fluorescent dye, reveals yellow bits of RNA inside spherical green vesicles.

with potential applications as sensitive sensors of biowarfare germs or as medical diagnostic tests. Other ribozymes have shown promise in fighting cancer, heart disease, and HIV. RNA organisms could evolve new ribozymes as well and also produce them in bulk as they multiplied. “Here we have a simple replicating nanosystem,” says Szostak. “Why not direct it to do useful things?”

That prospect lends a profound irony to Szostak’s quest. In trying to re-create the oldest life on Earth, he may end up spawning something entirely new. “There will probably be things to do with this system that we can’t even think of yet,” he says.

Szostak’s first step is to get a more sophisticated RNA molecule into the vesicles. He and his team hope to prove that a ribozyme can carry out real biochemistry inside a vesicle—even if that biochemistry consists of just cutting another RNA molecule in two. If they can pass this benchmark, their success will raise the odds that they’ll be able to make a replicase work inside the vesicles. “Once we have a real replicating RNA system and a real replicating vesicle system, we can put them together and really watch this system start to evolve,” Szostak predicts. “If the adaptive process is fast enough, it will be really fun to see how this system starts to become more complex.”

Watching the evolution of RNA-based organisms could tell scientists how life got its start on Earth. At the same time, it could alter the way scientists look for life on other planets and moons. The current strategy of astrobiologists is to look for signs of DNA-based life. That’s logical because DNA-based life is the only sort we know actually exists and the only sort scientists can study. But just because DNA-based life is the only sort on Earth today doesn’t mean it’s the only kind in the universe. Creating RNA-based life would show that alternatives are possible. “Once there’s one example of a lab system that’s evolving by itself, then the challenge is to build systems that can evolve under different conditions,” says Szostak. “Could we design cells that grow in environments without water?” Beyond Earth, liquid water seems to be rare. The most common liquid in the solar system is high-pressure liquid hydrogen in the giant gaseous planets Jupiter and Saturn. Could life exist there as well?

As Szostak and other scientists move closer to making new life, they inspire a lot of hand-wringing. Ethicists, philosophers, and theologians have weighed in. Environmentalists have warned of a Pandora’s box waiting to be opened. When asked about these issues, Szostak—understated as always—blinks his eyes slowly and gives a slight shrug. “This thing will basically have no biochemistry,” he says. “It won’t be able to live outside the lab.”

Nonetheless, Szostak suggests that the discoveries made by his research team could someday become a source of new kinds of biotechnology. There are already some companies dedicated to bringing ribozymes from the laboratory to the commercial world,