RNA World 2.0

Most scientists believe that ribonucleic acid played a key role in the origin of life on Earth, but the versatile molecule isn't the whole story.

BY JEF AKST

The ubiquity and diverse functionality of ribonucleic acid (RNA) in today's world suggest that the information polymer could well have been the leading player early on in the establishment of life on Earth, and, in theory, it's a logical basis for primitive life. One can readily imagine that RNA, as a catalytic molecule capable of serving as a template for its own replication, might have reproduced itself and grown exponentially in the primordial environment. Perhaps such an RNA-based proto-life-form even replicated with an appropriate level of fidelity to allow natural selection to begin directing its evolution.

But there's a snag: "The odds of suddenly having a self-replicating RNA pop out of a prebiotic soup are vanishingly low," says evolutionary biochemist Niles Lehman of Portland State University in Oregon.

For decades, researchers from diverse fields have theorized—and argued—about how early life might have begun, and about what sparked the 3.5 billion years of evolution that led to the plethora of cell-based life that occupies almost every nook and cranny of modern Earth. Different camps emerged. So-called "metabolism first" researchers focus on understanding chemical cycles that may have materialized in a prebiotic environment and could have led to the synthesis of nucleotides and other organic molecules. Those subscribing to the theory of "genetics first" want to identify the first information molecule and understand how it arose, replicated, and evolved.

The RNA world, first posited by Francis Crick[1] and others in the late 1960s, remains an attractive hypothesis. Many of the chemical hurdles that once challenged the laboratory synthesis of the molecule under presumed primordial conditions are being overcome, and in vitro evolution experiments are yielding RNA molecules that perform numerous functions, including copying themselves or other RNAs. "I don't think there can be much doubt that RNA was a major central player as both a catalyst and an early replicator," says Nick Lane, a biochemist at the University College London whose research falls under the "metabolism first" label. "So the RNA world is absolutely correct, as far as I'm concerned, in that."

WHAT DID FIRST LIFE LOOK LIKE?

"I really think that the crucial step, where I would say that these molecules became lifelike, is when two types of polymers cooperated with each other."

—Nicolas Hud, Georgia Tech

© KEVIN HAND

But the notion that RNA, on its own, spontaneously assembled and evolved on early Earth has fallen out of favor. More likely, whatever conditions spawned compounds as complex as nucleotides also generated other organics, perhaps early forms of modern amino acids and fatty acids, the constituent parts of proteins and membranes. "I'm not sure how many people anymore believe in a pure RNA world. I certainly don't," says Lane. "I think the field has drifted away from that, and there's now an acknowledgment it had to be 'dirty.'"

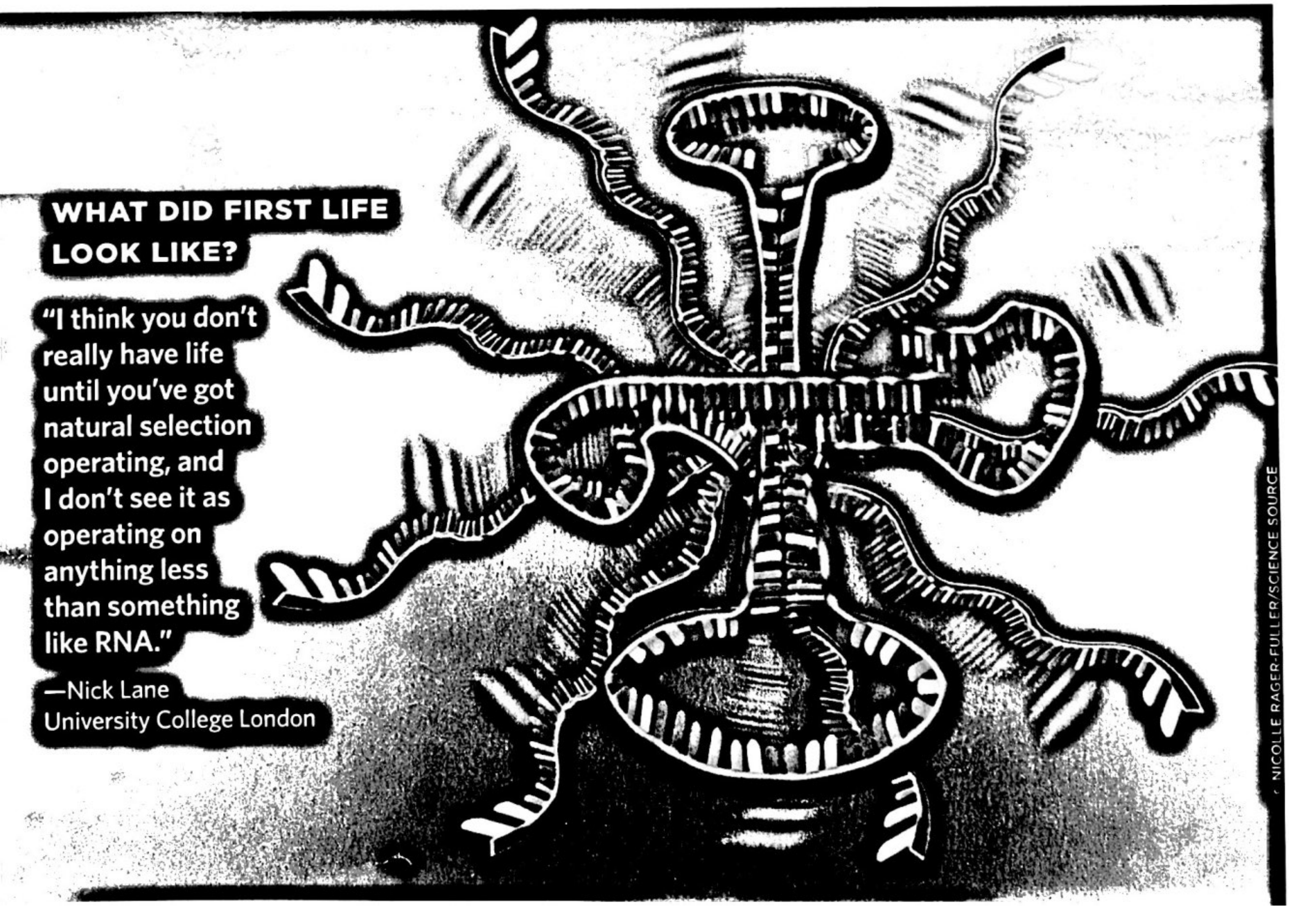
"I think most people would argue that there's . . . more than just RNA," agrees Matthew Powner, a "genetics first" origins-of-life researcher, also at University College London. (See "Matthew Powner: Origin Solver" on page 59.) "People have relaxed their opinions of the RNA world . . . from its original inception where RNA was fundamental to all parts of biology in the earliest form of life."

Spontaneous synthesis

RNA is suspected to have been an early lifelike molecule on Earth in part because of its supreme importance to modern life. RNA polymers carry DNA's genomic messages out of the nucleus and into the body of the cell, where they are used to assemble strings of amino acids. The ribosome, the critical piece of cellular machinery that translates those RNA messages into life-sustaining proteins, is, at its core, itself composed of RNA. And ATP, the universal energy currency in the cell, is a slightly modified RNA monomer.

But a long-standing weakness of the RNA-world hypothesis has been the inability to spontaneously generate the molecule's component nucleotides from the basic ingredients presumed to be available on the prebiotic Earth. Still today, "nobody has made all four of the nucleotides from one pot of simple starting materials," says Georgia Tech biochemist Nicholas Hud.

In particular, ribose, the five-carbon sugar that constitutes RNA's backbone, is difficult to form under prebiotic conditions, and purine and pyrimidine nucleobases, the variable parts of nucleotides, do not efficiently form covalent bonds with ribose. (See illustration on opposite page.) Myriad simulations in the lab, however, have yielded some promising answers. In 2009, for example, John Sutherland of the MRC Laboratory of Molecular Biology in the U.K. and colleagues demonstrated the formation of the pyrimidine nucleotides, cytidine (C) and uridine (U), from a handful of plausible prebiotic molecules under conditions consistent with current early-Earth geochemical models.[2] Rather than rely on free ribose and nucleobases, the team sequentially derived the complete ribonucleotides from glycolaldehyde and glyceraldehyde—"the smallest molecules you might consider sugars," explains Powner, a collaborator on the study. And



WHAT DID FIRST LIFE LOOK LIKE?

"I think you don't really have life until you've got natural selection operating, and I don't see it as operating on anything less than something like RNA."

—Nick Lane
University College London

NICOLLE RAGER-FULLER/SCIENCE SOURCE

in September 2012, Sutherland showed that these sugar building blocks could be derived from hydrogen cyanide, a suspected prebiotic molecule important in synthesizing amino acids.[3]

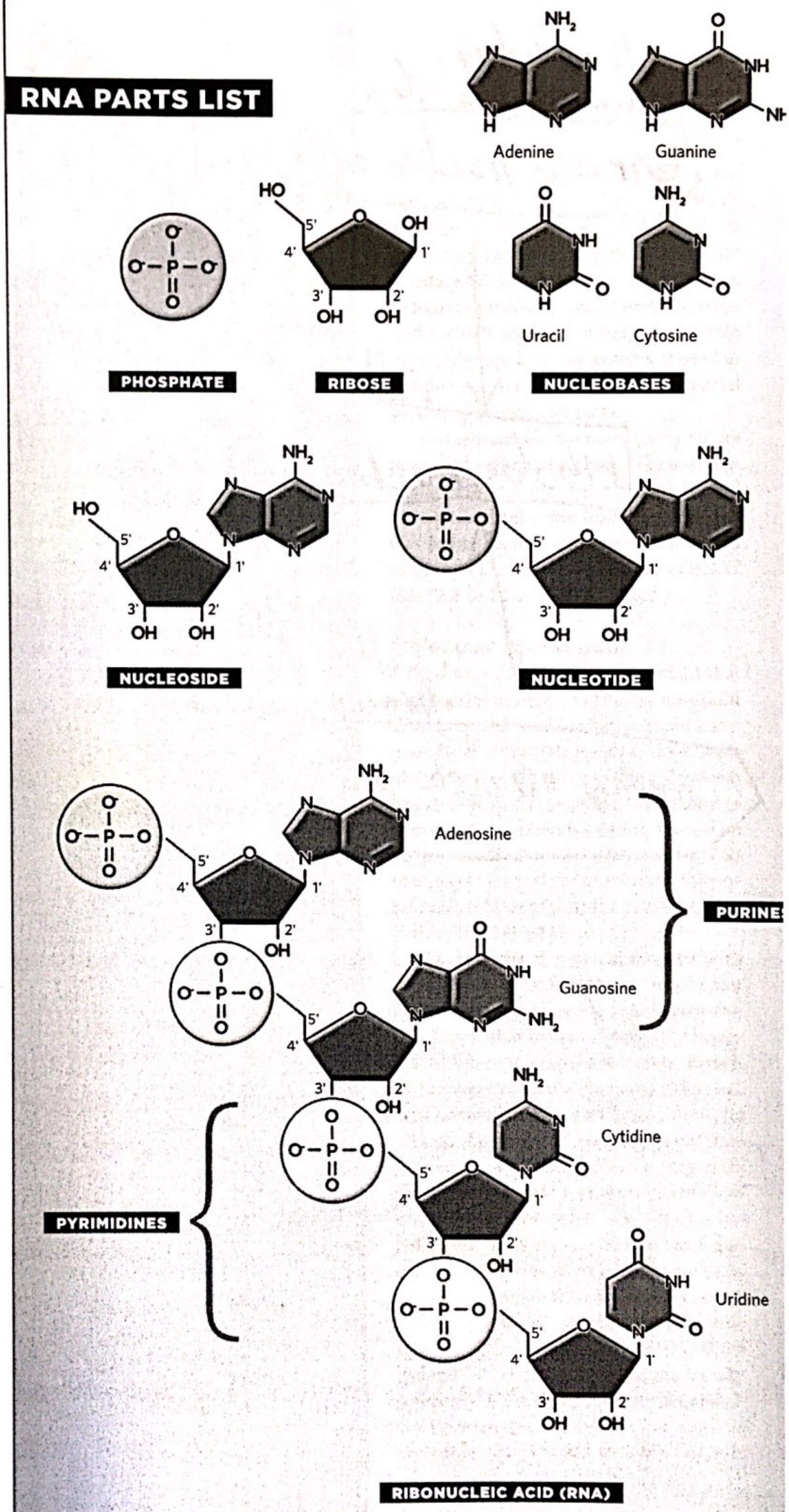
Scientists have yet to produce the purine nucleotides adenosine (A) and guanosine (G) under similar prebiotic conditions, but the research is moving in that direction, says Powner. "There's nothing I see, other than time and effort and a few bright ideas, that stands in the way of understanding at least [the] chemistry to the monomeric components of biology," he says.

Powner and others are now turning to a different challenge: how those nucleotides link up into a molecule even a fraction as complex as modern RNAs. "How [do] you control polymer synthesis, polymer length, the interaction of macromolecular structures? And how [do] you make things that will specifically function as polymers, without getting a statistical mess?" Powner asks. "That's where I see the synthetic area of this chemistry at the moment."

One challenge of RNA polymerization is that there isn't just one way for two nucleotides to bind. The phosphate group can link the 5' carbon molecule of one sugar with either the 2' carbon or the 3' carbon of its neighbor. In life, thanks to the oversight of RNA polymerase, all RNAs are assembled by 3'–5' phosphodiester linkages. But when generating RNAs in vitro, researchers get a mixture.

Last April, Powner, Sutherland, and their colleagues published evidence that a chemoselective acetylation process could support the generation of 17-nucleotide-long RNA molecules with predominantly 3'–5' linkages under prebiotic conditions.[4] In the same issue of *Nature Chemistry*, Powner and other colleagues also showed that the presence of a mixture of different RNA linkages within a polymer didn't matter: it did not disrupt the folding of the molecules, nor their catalytic functions.[5]

Meanwhile, Lehman's group is unearthing evidence that if some small RNA polymers did arise, they may have had a fighting chance. Short oligomers


RNA PARTS LIST

of RNA, approximately 50 to 100 nucleotides long, are capable of recombining, bringing together different RNA units.[6] "So as long as there's an abiotic mechanism for producing small pieces of RNA, if you can recombine those pieces together, you can start building up your repertoire of catalysts," Lehman says. And last year, he found that RNA fragments can be recycled,[7] which could have helped generate the ample supply of nucleotides needed to support the replication and exponential growth of genetic elements.

To Lehman, the chemical pieces of the puzzle are falling into place. "I'm optimistic that within 5 or 10 years, we will indeed have a chemical route from the stuff that was laying around on the prebiotic earth to RNA or something quite close to RNA." (See "A Pre-RNA World?" at left.)

Molecular cooperation

Of course, a primordial environment that could support RNA synthesis no doubt also spawned many other organic compounds—for example, peptide- and lipid-like molecules, which are chemically much less challenging to generate. "It's absurd to think that you might have some kind of an environment where you have just a load of nucleotides or RNA in solution, uncontaminated by anything else," says Lane at University College London.

These molecules would interact with each other, and though they would not have been self-replicating or able to evolve in a Darwinian fashion, one could imagine a different kind of selection driven by the kinetics of the chemical reactions, says Lehman. Under these conditions, he argues, the notion of early life being contingent on a selfish molecule may miss the mark. When it comes to mixtures of interacting molecules, the Darwinian concepts of individual fitness and discrete generations do not apply, he says. "As you approach the origin of life itself, you have to think outside the box a little bit to imagine these systems getting off the ground. . . . The rules of the game are significantly different in the first moments."

Lehman and others suggest a sort of molecular cooperation as a key factor in

VOSSMAN/WIKIPEDIA

A PRE-RNA WORLD?

Even if biochemists conclusively demonstrate the plausibility of RNA arising on a prebiotic Earth, that doesn't mean it did. Georgia Tech's Nicholas Hud, for one, believes that other biomolecules probably preceded RNA, and that RNA itself is a product of evolution.

In addition to the continued challenges in the prebiotic synthesis of RNA, Hud argues that RNA's extremely good fit to its contemporary catalysis roles suggests some sort of selection process was at play in the earliest stages of these chemical life forms. "RNA is just absolutely perfect in what it does today," Hud says. "To me, that's the hand of evolution." And why not? After all, some believe DNA evolved from RNA to take its place as the center of the modern genetic universe.

One of the earliest proposals that the first RNAs might have looked a little different came in 1987, when Stanley Miller, Gerald Joyce, Leslie Orgel, and Alan Schwartz—all respected pioneers in the study of the origins of life on Earth—proposed that a pre-RNA molecule might have had a different backbone (*PNAS*, 84:4398-402, 1987). It was a key moment for the field, Hud says. "That's where I think we started relaxing this opinion that [for life to start] we need to have molecules that we have today."

One promising molecule that has been studied to date is threose nucleic acid, or TNA, an RNA-like molecule with a backbone composed of a four-carbon sugar called threose, which is much simpler to make under prebiotic conditions than ribose. "[Threose] can be synthesized in a single step from the same two-carbon subunit," says John Chaput of the Biodesign Institute at Arizona State University. "And this idea of chemical simplicity is really attractive." Indeed, threose is more common than ribose on meteorites. Moreover, Chaput adds, "not only do [TNA polymers] base pair with themselves, but they base pair with RNA. So that means that if they did precede RNA in the origins of life, they have at least a mechanism for transferring information onto RNA."

Another RNA-like molecule that researchers have derived in the lab is phosphoramidate DNA, which is constructed from amino nucleotide building blocks that "are so much more reactive than ribonucleotide building blocks that the polymerization goes quite well," says Jack Szostak of Harvard Medical School and Massachusetts General Hospital. But experimental work on such alternative polymers lags behind that on RNA, and thanks to John Sutherland's work on the prebiotic synthesis of RNA, Szostak is not convinced that anything did precede the modern molecule. "I really do think [RNA] is the best bet now for the first genetic polymer," he says.

Others reserve judgment. "I use RNA as a model system because it works so well," says Portland State University's Niles Lehman. "Whether there was something that preceded RNA that was a lot like RNA, I'm not going to take a stand on."

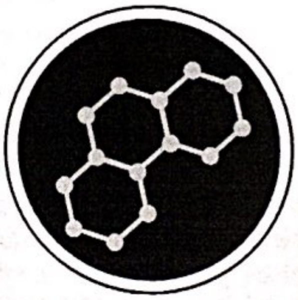
the origin of life. Polymers that assembled with other polymers might have been better protected against hydrolysis, for example, and as a result, started growing in number. Over time, these chemical systems could have "evolved" to be more stable and more complex. As more species of molecules joined the interactions, they may have created chemical networks that began to take on functions. "Imagine that, of [a] set of molecules, there might be some that catalyze a chemical reaction that gives rise to a molecule that's needed—something that's in short supply, for example," says Hud. "That would then allow the polymers that are around this 'generator' to increase in number. And so there's a functional sequence."

To understand the earliest stages of these chemical systems on Earth, a handful of researchers are drawing inspiration from a particular type of deep-sea thermal vents that are alkaline and not too hot. Michael Russell of Caltech's Jet Propulsion Laboratory in Pasadena, California, predicted that conditions inside such vents would make them favorable sites on early Earth for the abiotic beginning of life. Lane describes the microporous matrix of the vents, through which alkaline fluids chock-full of hydrogen gas flow. Conveniently, these solutions will cluster organic compounds through a process known as thermophoresis. "It's about the only kind of system I can think of, from a theoretical point of view, which has all the chemical and thermodynamic conditions right that it can produce organics continuously and concentrate them," says Lane.

Moreover, in the acidic oceans of young Earth, with CO_2 levels anywhere from 10 to 1,000 times higher than today's oceans, such alkaline fluids may well have generated natural proton gradients similar to those that drive ATP production in modern organisms, says Lane. Both Lane and Russell have built prebiotic vent-simulating bioreactors to test these ideas.

Not wanting to limit his search to a subset of geochemical conditions, Powner is taking a different approach: explore all possible chemistries for common conditions that may have yielded the different components of the first molecular systems. "If we want more than RNA, because a plausible living system would likely incorporate more than RNA, we're probably going to need some form of compounds relating to amino acids, some kind of membrane-forming compounds, something that can recruit and capture energy," he says. "We need to understand how we can not only build these components, but build them all together."

One molecule type that has held the attention of Nobel laureate and Harvard Medical School biochemist Jack Szostak for more than a dozen years is fatty acids, which, like the phospholipids of modern cell membranes, have a hydrophobic tail and hydrophilic head. Szostak and others have suggested that genetic molecules and fatty acids might have worked together "to get Darwinian evolutionary processes going that would lead you on a path to modern life," Szostak says. In vitro work over the past decade has yielded fatty-acid vesicles that can grow and divide under prebiotic conditions, and researchers have even begun to combine these replicating vesicles with genetic elements. "Of course the ultimate goal is we want to have a replicating nucleic acid inside replicating vesicles," says Szostak, who also holds a position at Massachusetts General Hospital.



WHAT DID FIRST LIFE LOOK LIKE?

> **"A self-sustaining chemical system capable of evolution. If I'm in a dark alley with a gun to my head, that's the definition I'm going to give."**
>
> **—Niles Lehman**
> **Portland State University**

An enduring challenge to this achievement, however, has been magnesium, which is necessary for RNA polymerization but causes fatty acids to precipitate out of solution. "For years, that was a big roadblock," says Szostak. But last November, he and his colleagues came up with a solution: by adding citrate to the mix, the team was able to prevent fatty-acid precipitation while allowing RNA chemistry to proceed as it should.[8] "So for the first time we were able to do template-copying experiments where the [RNA was] inside a fatty acid vesicle," he says. "That's a big step towards having a complete protocell model."

As far as pinpointing when "life" emerged from the molecular activity of early Earth, many argue that the endeavor is a bit of a red herring. "Nobody can define what life is, and it's a pointless question," Lane says. "Is a virus alive or not? What about a retrotransposon? It's a continuum between nonliving and living. People tend to draw a line across that continuum which reflects their own interests."

"[It] is pretty arbitrary," agrees Szostak, who in 2012 published a short comment on how attempts to define life do not inform the search for its origins.[9] "So rather than waste time on a sterile debate, I'd rather just get more experimental work done to try to fill in the gaps in our understanding . . . from planet formation through simple chemistry on a young planet, up to more and more complicated chemistry, and then up to the first cells."

What RNA can do

In the early 1990s, with the invention of in vitro evolution techniques that supported the "breeding" of RNAs in a test tube, the race was on to see what the molecule was

WHAT DID FIRST LIFE LOOK LIKE?

> **"It's hard to define life, a satisfying definition for life, but basically all of them, I think, would have the word evolution in them. If you don't have a system that is capable of Darwinian evolution, then it's hard to make an argument that it's a living system."**
>
> —Michael Robertson
> Scripps Research Institute

capable of. Researchers directed the evolution of RNAs that could catalyze monomer synthesis, from the production of ribose to the attachment of the sugar to nucleobases. Others bred RNA enzymes, or ribozymes, that could conduct the steps of translation, phosphorylate other polymers, join molecules together, or break them apart. "Of course the big [function] is the one that Francis Crick talked about"—self-replication, says Gerald Joyce of the Scripps Research Institute in La Jolla, California, who helped pioneer in vitro evolution techniques. "Imagine if you had an RNA enzyme that had the function of producing copies of a parent RNA molecule, including itself."

At the turn of the century, Joyce and Scripps colleague Natasha Paul hit the jackpot. In a 2002 *PNAS* paper, they described an RNA molecule that could, for all intents and purposes, make copies of itself.[10] It wasn't complicated: a ribozyme that the researchers dubbed "E" joined together two component RNA pieces, "A" and "B;" when ligated, "A" and "B" made "E."

It didn't do much other than self-replicate, Joyce admits, but it suggested the possibility that RNA could, without experimenter intervention, evolve. "We don't have the smoking gun of some RNA-based life form out there . . . [and] we don't have direct fossils of the RNA world," he says. "So then you fall back on: What can we make in the laboratory to teach us about what RNA can do?"

Joyce's lab went on to develop a cross-replicating ribozyme system, in which each of two different small RNA molecules made copies of the other. And with a bit more directed evolution, Joyce's PhD student Tracey Lincoln was able to improve the system's kinetic properties such that it began replicating exponentially.[11] "There literally was a day when the thing went critical," Joyce recalls.

"This system is unique in the sense that it's currently the only RNA system that replicates exponentially," says molecular biologist Michael Robertson, a staff scientist in Joyce's lab. "So there's all kinds of different evolutionary experiments that you could imagine doing."

Most recently, Joyce and Robertson evolved what they call the super-replicator, which can undergo 10^{100}-fold amplification in 36 hours, doubling every five minutes.[12] The replicator Joyce developed with Lincoln doubles only once every 30 minutes. "So this thing really cranks," Joyce says of the super-replicator. "And that's letting us now do more powerful test-tube evolution."

Of course, these artificial systems are unlikely to resemble the first RNAs to appear on the young planet, Joyce notes. "There was no Tracey [directing evolution] on the primitive Earth. This is not that kind of game." Devising a self-sufficient RNA system could nevertheless be informative "of the later stages, perhaps, of some kind of RNA world scenario," says Robertson, who is now trying to evolve the ribozyme system to do something besides replicate.

Joyce admits to another motivation for his research: he's hoping to beat the astrobiologists to the discovery of a new kind of life. "I think there's a pretty good shot at making RNA-based life from scratch, even if it never existed on Earth, just to make a second life-form." ■

References

1. F.H.C. Crick, "The origin of the genetic code," *J Mol Biol*, 38:367-79, 1968.
2. M.W. Powner et al., "Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions," *Nature*, 459:239-42, 2009.
3. D. Ritson, J.D. Sutherland, "Prebiotic synthesis of simple sugars by photoredox systems chemistry," *Nat Chem*, 4:895-99, 2012.
4. F.R. Bowler et al., "Prebiotically plausible oligoribonucleotide ligation facilitated by chemoselective acetylation," *Nat Chem*, 5:383-89, 2013.
5. A.E. Engelhart et al., "Functional RNAs exhibit tolerance for non-heritable 2'-5' versus 3'-5' backbone heterogeneity," *Nat Chem*, 5:390-94, 2013.
6. E.J. Hayden et al., "RNA-directed construction of structurally complex and active ligase ribozymes through recombination," *RNA*, 11:1678-87, 2005.
7. N. Vaidya et al., "Recycling of informational units leads to selection of replicators in a prebiotic soup," *Chem Biol*, 20:241-52, 2013.
8. K. Adamala, J.W. Szostak, "Nonenzymatic template-directed RNA synthesis inside model protocells," *Science*, 342:1098-1100, 2013.
9. J.W. Szostak, "Attempts to define life do not help to understand the origin of life," *J Biomol Struct Dyn*, 29:599-600, 2012.
10. N. Paul, G.F. Joyce, "A self-replicating ligase ribozyme," *PNAS*, 99:12733-40, 2002.
11. T.A. Lincoln, G.F. Joyce, "Self-sustained replication of an RNA enzyme," *Science*, 323:1229-32, 2009.
12. M.P. Robertson, G.F. Joyce, "Highly efficient self-replicating RNA enzymes," *Chem Biol*, 21:1-8, 2014.