How to Build a Living Thing

by

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ABSTRACT

A number of research groups worldwide are working on various aspects of the problem of building life from scratch. Jack W. Szostak's lab in Cambridge, Massachussets is one of the centers of the action. Open a recent news article on some discovery related to synthetic life or life's origins on Earth, and he's likely to be quoted. Szostak fills his lab with ambitious, bright, young people, a few of whom have gone on to found their own labs. His work provides a lens through which to view the contemporary state of progress toward the ancient and ambitious goal to take what was not alive before and make it live. Starting from an initial plan to make a self-assembling, self-replicating membrane containing a self-replicating genetic molecule, the lab has had some striking successes and, off course, some setbacks. Recent breakthroughs suggest that the realization of a wholly human-designed and created life form looms in the foreseeable future.

Thesis Advisor: Tom Levenson
Title: Director, Graduate Program in Science Writing

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How to Build a Living Thing

A translucent hairy pickle, visible only under the microscope, *Tetrahymena thermophilia* spends its days floating in temperate bodies of freshwater, motoring around with its thousands of tiny hairs, snacking on passing bits of debris. Most people wouldn't distinguish it from pond scum because it is, in fact, pond scum.

To scientists, however, this little protozoan has yielded critical secrets about how life works. In the early 1980s it told a young researcher at the University of Colorado named Thomas Cech what might turn out to be the biggest one of all. It used to be assumed that cells observed a strict division between Labor and Management. DNA knew what to do; complex molecular machines called ribosomes knew how to do it. In 1980, everybody knew that RNA was just a messenger, a molecular errand-boy relaying instructions from DNA in a cell's nucleus to the ribosomes in charge of making the larger molecules required to keep a cell alive. The intricate work of the cell was handled by protein enzymes, twisty, folded molecules whose physical shape allows them to facilitate otherwise unlikely chemical reactions.

But with *T. thermophilia's* help, Cech discovered that such strict division of labor did not always hold in nature. He found that some strands of *T. thermophilia's* RNA could snip off pieces of themselves without the help of proteins. This humble reaction turned molecular biology on its head: now enzymes weren't limited to being made of proteins, they could be made of RNA. Cech and Sidney Altman—who found a similar phenomenon at Yale—won the 1989 Nobel Prize for Chemistry, for this discovery.

With the ability to both store information and catalyze reactions, the possibility arose that RNA might be a biological entrepreneur, simultaneously directing and carrying out the functions of a simple cell. Life based solely on RNA became a feasible idea, though it had never been observed before in nature. Many began to believe that RNA-based life might have preceded the current world of DNA, RNA, and proteins. Nobel Laureate Walter Gilbert coined the term "RNA world" in 1986 to describe such a primordial Earth.

Origins researchers imagined seas, or at least pools, of RNA molecules having different properties, undergoing a form of molecular evolution. Some molecules might be active enough to make copies of themselves. These would have an advantage, population-wise, over molecules that couldn't. Mutations that led to better replication would drive evolution and set in motion the chain of events that billions of years later would lead to dogs and human beings.

The problem is that all of this would have happened somewhere around 3.5 to four billion years ago. Scientists believe that fossil evidence of the event would have long been destroyed. This leaves two options for anyone interested in figuring out how life might have started on Earth. The first is to look at the geological record and speculate about what chemicals might have been present, what the early oceans were like, what the atmosphere was like, and imagine possible chemical routes to life. The second is to put on the lab coat and safety goggles and actually try to make it happen right now.

Cech's discovery of ribozymes hinted that this second approach might be feasible. A little less than twenty years after the breakthrough, a group of scientists who had been working on origins of life problems, Jack Szostak of the Howard Hughes Medical Institute, David Bartel of MIT's Whitehead Laboratory, and Pier Luigi Luisi of the Institut für Polymere in Switzerland set out to see just how powerful a ribozyme might be. They called their shot in a January 2001 issue of the journal *Nature*, laying out a roadmap to build a simple primitive cell—a protocell. The paper was called "Synthesizing Life", and in it they claimed it would be possible for humans to do what had never been done before: build a living thing, from scratch, in the laboratory.

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A number of research groups worldwide are working on various aspects of the problem of building life from scratch. Szostak's lab in Cambridge, Massachussets is one of the centers of the action. Open a recent news article on some discovery related to synthetic life or life's origins on Earth, and he's likely to be quoted. He fills his lab with ambitious, bright young people, a few of whom have gone on to found their own labs. His work provides a lens through which to view the contemporary state of progress toward the ancient and ambitious goal to take what was not alive before and make it live.

Sitting in his wood-paneled office, Jack Szostak's calm open-ness conceals a core of ambition and brilliance. With glasses and a roundish face—Carl Zimmer once wrote that he looks like an "affable owl"—he's quick to smile and is commonly referred to by students and colleagues as "great." He's a scientific jack-of-all-trades, "If you want to do a project like this, you have to be willing to go from molecular biology to biophysics to organic chemistry, just do whatever's necessary," he said.

Szostak is fascinated by the question of how life began on Earth, but in his current research, he does not intend to retrace the steps that life actually took. More than that: he is doubtful that anyone can prove the exact sequence of pre-historical events. Instead he would be very happy at his stage to build any sort of chemical system that might be considered alive.

His roadmap paper in *Nature* laid out the essential task plainly: "*The first challenge on the path to a synthetic life form is to imagine a collection of molecules that is simple enough to form by self-assembly, yet sufficiently complex to take on the essential properties of a living organism.*"

Simple and complex: a yeast cell, one of the simplest single-celled organisms, has about 3,000 genes, each one encoding the instructions needed to build a protein. The proteins in turn interact with each other and smaller molecules, sugars, for example, to carry out chemical reactions, like respiration or protein synthesis, required to keep the yeast cell alive. With millions of moving parts, each carrying out a delicate yet necessary task, a modern living cell is masterpiece of complexity, well beyond what a scientist could hope to design from scratch. Presumably, however, life wasn't always this complicated.

Assuming that a living system had to at some point be simple enough to form on its own from pieces that were not alive before, Szostak, Bartel, and Luisi broke the problem down further, "*We can consider life as a property that emerges from the union of two fundamentally different kinds of replicating systems: the informational genome and the three-dimensional structure in which it resides*."

Reducing the larger problem of life to the two comparatively simple problems of a self-replicating genome—presumably a bit of RNA to drive cellular reactions and pass information to future generations—and a self-replicating 3D structure—to keep the system together—provided a glimpse of the way forward. Inspired by Cech's work, a functioning information molecule would serve as a proof of concept for the RNA world. The structure could keep the rest of world out of a fragile replicator's business. Together they would form a system that might have a fighting chance of being called "alive".

Building each piece would not be easy, and even if it were, there was still a big catch. Not only would the pieces have to replicate themselves, they would have to be able to evolve. Changes in the RNA would have to be able to give the protocell an advantage over other protocells, and those changes would have to be heritable, so that subsequent generations could accumulate and capitalize on helpful mutations. None of these problems had been solved at the time.

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Jack Szostak's lab occupies about half of the seventh floor of the Simches Medical Research Building at Massachusetts General Hospital. The mirrored-glass encased building is home to a number of medical research labs. From the inside, Szostak's lab looks as one might expect a chemistry lab should. Lab benches seem to stretch to infinity, separated by shelves filled with beakers, boxes of rubber gloves, and a menagerie of chemical jars made of dark brown glass or white plastic with red lids.

Grad students and post-docs clad in street clothes hunch over laptops and brown lab notebooks filled with light-green graph paper. Others in white lab coats and sleek safety goggles stand at benches and chemical hoods, holding up the occasional small clear-plastic vial or half-liter-sized round flask, seemingly always with a pipette in the other hand. Under the hoods, larger glass flasks, beakers, and convoluted systems of glass and rubber tubes sit in various stages of chemical reactions.

The Szostak, Bartel, and Luisi approach to protocells was, and is still, to make some sort of self-replicating information molecule and stick it inside of a self-replicating membrane. In other words, put information into a sack. Reflecting this plan, the lab is divided more or less into two groups. One team is working on membranes. The other works on genetic molecules. "Having put out those ideas, I felt we should, you know, give it a try," said Szostak. "And not just be one of these people who puts out theories and then lets them lie."

The membrane is crucial for two reasons: materials and protection. To make copies of itself, the information molecule needs to have ready access to simpler molecular building blocks. A membrane can trap the necessary small molecules. A would-be replicator also needs protection from other information molecules, molecular chop-shops that might disassemble it for spare parts. A barrier of some sort solves both problems, providing a vessel to gather and concentrate material for reproduction while keeping away the moochers. To design his protocell, Szostak drew inspiration from the most obvious natural example, the cell membrane.

Natural cell membranes are composed chiefly of lipids, the group of molecules that includes fats and oils. Lipids are basically a chain of carbon atoms with a head made of oxygen and hydrogen. The head is often hydrophilic—meaning that it enjoys the company of water molecules. The tail is the opposite, hydrophobic. Because of this dual preference, lipids have a tendency to self-assemble in useful ways.

Under some conditions, the hydrophobic tails of a bunch of lipid molecules will congregate together, forming the center of a ball whose surface is made of the hydrophilic heads. In diagrams, these *micelles* look a bit like a fistful of lollipops, arranged with the candy outwards.

Other conditions lead the lipid molecules to assemble into two-layered sheets, the lollipop tails sandwiched between the candy heads. If the sheets are large enough, they can curve around and form a hollow sphere, a *vesicle*. Whether the lipids form a micelle or vesicle depends on the pH of their environments—the amount of hydrogen ions floating around in the water. Higher pH, more basic solution, leads to micelles; lower pH, or more acidic solution, leads to vesicles. Go too low, however, and the vesicles give way to oily blobs.

With nothing but water inside initially, vesicles provide a simple, self-assembling container in which a would-be replicating molecule might enjoy safety and a place to collect the materials it needs. If the whole system is to come "alive" however, the membrane, along with the information molecule, must grow and eventually divide.

Prior to the 2001 roadmap paper, Pier Luigi Luisi had shown that a vesicle sitting in water with a bunch of micelles will gradually steal lipids from the micelles and incorporate them into its membrane. This steady accumulation leads the vesicle to expand and was for a time the only known process by which vesicles could grow. Then one of Szostak's students, Irene Chen, found a completely different way. Vesicles that have a lot of RNA in them, can steal membrane molecules from vesicles that are not so full of RNA. "So they can grow at the expense of their neighbors," said Szostak. Chen's discovery showed that the growth problem had at least two different solutions and hinted that some protocells might have an advantage over others.

With two ways to grow vesicles in hand, Szostak shifted focus to the tougher problem of division. When he proposed that his protocell have a lipid vesicle membrane, no one had yet figured out how it might divide. "You've gotta have purely physical-chemical ways of making this cell envelope grow

spontaneously and divide, without any biological machinery," Szostak explained. "We didn't really have any mechanisms that would do that."

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On the shelf behind graduate student Rafael Bruckner's desk, taped above cutouts of paintings of birds and random Korean newspaper advertisements, is a certificate from Newton Elementary School for outstanding 4th grade achievement in Biology. Bruckner is blonde with blonde eyebrows and wears skate-style shoes and a dark zippered hooded sweatshirt. As a Ph.D student in Szostak's lab he's worked on the problem of vesicle replication since 2006.

In the past year, Bruckner has been developing models for how the lipid molecules in a vesicle behave when a vesicle is forced through a microscopic hole. This method of membrane division, called extrusion, was the Szostak lab's first solution to the division problem. "Just force a big vesicle through small holes and little vesicles come out the other side," Szostak said.

Szostak and colleagues published this extrusion method in 2003 and since that time have been studying the details of how the lipids behave, hoping to get insight into a more natural, less forced, mechanism for cell-division. The approach is characteristic of the Szostak method: look at any example that works to gain insight into better ways.

Bruckner has developed his model of the extrusion process to the point where he is ready to compare it against how actual vesicles behave. To do this, he needs to make vesicles, force them through a tiny opening, and catch the whole thing on camera so that he can analyze what happens.

Making vesicles, he claims, is relatively easy. Basically just add olive oil to water and shake. The actual process is a bit more precise and uses clean containers and special shakers to agitate the mixture. The product is an inch-long plastic vial filled with clear liquid made slightly cloudy by the hundreds of thousands of microscopic vesicles floating in solution.

He needs only small vesicles for the experiment; large ones will clog his instruments. The ideal size is no larger than ten microns in diameter. Ten vesicles of this size, laid side-by-side, would stretch the width of a human hair. They have to be this small because Bruckner will be running them through a microfluidic device—a network of tiny tubes and channels that can direct fluids with precision. His current set-up consists of a slide-thin glass rectangle about the size of a playing card with two, inch-long cones of epoxy glue standing up, each with a strand of clear spaghetti-thin tubing coming out the top.

With clenched fists, he pushes the plunger on a syringe connected to one of the tubes while water dribbles out of the other one into a clear plastic beaker. After flushing the device, he pinches off the tubes with regular black binder clips to keep the newly-cleaned instrument pristine.

He brings the rig into a messy, closet-sized, microscope room, hooks up his laptop to the scope and within a couple minutes brings up a display of the

inside of the device. Pointing to a large, magnified, hollow needle on the left hand side of the screen, he explains, "hopefully the vesicles that we filtered are going to shoot out through here."

He motions across the screen to point to another needle, entering from the right side of the screen, perhaps one-twentieth the thickness of the larger one.

"They'll then be forced through this tiny channel," he says.

He starts a pump that pushes the vesicles through the device. "There…stuff's coming out." A stream of blurry translucent dots flows from left to right across the computer screen. The idea that any of the vesicles would enter the needle seems improbable to say the least. The whole process is a bit like trying to throw water balloons into a drinking straw.

He adjusts the contrast, inverting blacks and whites in the hope of seeing one of the blurry blobs go in the needle. "The vesicles, there's not much to them, you can't really see them unless you filter the light," he says. Water is collecting in the exit tube, so he knows that something, at least, is flowing through the target needle, but if it's vesicles, they have so far eluded detection.

Nothing seems to be happening, and it would be easy to miss if it did. For this reason he records the experiment with a high-speed video camera built into the scope. Needless to say, Bruckner's spent more than a few late nights watching blurry blobs travel across the screen. Today, after about twenty minutes of searching, he's ready to call it quits. This particular set up doesn't seem to have promise.

Back at his desk, he brings up video of a previous, similar yet successful experiment. He points to a circular blob on the left side of the screen. "So this is a vesicle," he says, "this onion-type thing." The vesicle has multiple visible layers—most vesicles, it turns out, have this structure. Rare is the perfect, single layered membrane. In the frozen frame it hovers just outside a glass needle, width about half the diameter of the vesicle. The needle stretches three-quarters of the way across the screen.

He starts playback in slow motion and the vesicle attaches to the opening of the needle. Parts of the membrane visibly stretch down the length of the tube as the circular vesicle shrinks.

He brings up another movie—same setup, larger vesicle. This time instead of squeezing through to make one elongated tube, little vesicles pinch off and flow down the tube like bullets made of clear Jello.

"The difference between those two experiments is that in the previous one, the vesicle entered the channel symmetrically…here it's perched like this."

He makes a fist with his right hand to represent the vesicle and holds up a pen to represent the needle. His fist sits below the pen, demonstrating the spatial relationship of the needle and vesicle. The needle siphons off material from the membrane, sucking pieces into the tube. Surface tension then causes the newly formed babies to seal off.

It's replication, technically, but the researchers in Szostak's lab don't think its how an actual protocell membrane would reproduce. Spend enough time waiting for the little clear baggy to enter the little clear needle, and it's easy to see

why this probably isn't the way that life started, or even a particularly good method for a synthetic living thing to reproduce itself. It's too forced, not lifelike.

Another researcher in Szostak's lab, Ting Zhu, has what seems to be a more natural approach. Published in March of 2009, Zhu showed that as a spherical vesicle grows in the presence of lipids, it forms blobby, tubular, amoeba-like arms. The vesicle arms get longer and longer and become more and more unstable. Once they get long enough, any slight turbulence will cause them to snap off and reseal themselves. Zhu showed how this mechanism works and, importantly, that contents inside the original vesicle end up in the "daughter" vesicles with very little loss. This method of division seems more promising than extrusion because it is more natural; the vesicles do it on their own, with no forcing from outside, other than a bit of turbulence to destabilize things. Zhu's method could be a workable solution to the division problem that in 2001 seemed daunting.

"I think almost all problems seem complicated at the beginning when you don't understand how they possibly could work," said Szostak. "But now the growth and division aspect of the cell membrane seem pretty simple, it's easy to do, nothing fancy about it. It works with simple molecules." With two distinct methods for membrane division, a formerly formidable challenge now seems easy. "The real big problem," Szostak admits, "is just the chemistry of replication."

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A self-assembling, self-replicating membrane, while an important step towards life, would not be considered alive by most researchers. It lacks any obvious mechanism by which an individual membrane might acquire advantages over others, a requirement if the system is to evolve. The membrane needs some guts inside, stuff that does stuff. These guts not only have to give the system as a whole a potential advantage over others—for example, by catalyzing the formation of more membrane material—they also have to make copies of themselves that can pass along the advantage to future generations. Cech had shown that with the ability to both store information and catalyze reactions, RNA might be a good candidate.

Taking their cue from Cech, Bartel and Szostak tried to make an RNA molecule that could catalyze its own replication. Like a normal RNA molecule, it would store information in the form of a specific sequence of building blocks known as nucleotides. Like DNA, this sequence would serve as a template or set of instructions for making a copy of the molecule. DNA in a cell, however, has a set of protein enzymes that help to translate the instructions into a new copy of the molecule. Because protein enzymes require their own set of complicated molecular machinery to produce, an RNA replicator in Szostak and Bartel's very simple design would not have them around to help it make copies of itself. The RNA chain would have to take on this vital task by folding up into a physical shape that would then be able to take individual nucleotides and attach

them together. It would serve as both genome and enzyme, manager and chief employee, a ribozyme.

Cech had shown that ribozymes exist in nature, but none had been found that could execute reactions as complex as self-reproduction, making a complete, functional copy of itself. Szostak and Bartel knew that if their approach was to work as planned, they would need a way to overcome this complexity. "It became obvious that if we wanted to make more progress, we'd have to learn how to design or evolve our own ribozymes," Szostak recalled. "So that led me to a sort of whole new area of in-vitro evolution."

Szotak explained that the problem of designing a molecule with all the right chemical properties *and* that assembles itself into the right shape is too complex to work out from first principles. One can't just add together one atom after another, using the basic laws of chemistry. There are too many possibilities to try, too many variables to pin down. So he and Bartel deided to try to copy the way nature works: try everything at once and select out the designs that work best. This process draws upon the ideas of Leslie Orgel, a British organic chemist, famous for his work with self-replicating chemical systems. Orgel is credited with saying "Evolution is cleverer than you are."

Szostak and Bartel used this approach—evolution in a bottle—to uncover new promising molecular designs. The idea is to start with a more or less random strand of DNA or RNA and then use enzymes to copy it while simultaneously introducing mutations. The population of molecules can then be run through a test, such as bonding to a particular sugar, like galactose. The strands that bond strongly with galactose will be saved, those that don't, tossed. The saved molecules then go through the process again, accumulating advantageous changes until researchers end up with a molecule that does more or less what they want.

Ribozymes, like all enzymes, work because their three-dimensional shape allows them to ferry molecules around, moving them into positions where they are more likely to bond with the right other molecules. This physical arrangement is of prime importance. Determining a ribozyme's sequence of bases—the information stored within—is relatively easy, but figuring out its three-dimensional structure and how that structure will affect the way the molecule functions can be maddeningly difficult. In-vitro evolution worked wonders in this regard, giving Bartel and Szostak a way to select molecules with the functions they wanted without knowing beforehand what they would look like or how they would work. Szostak described that he and Bartel used this technique to "make new RNAs that did all kinds of interesting things."

The overall aim, however, was to evolve a piece of RNA that could function as a ribozyme that copies itself, known as an RNA replicase, and here too—they made progress. Starting with a completely random sequence of nucleotides and using in-vitro evolution, Bartel first was able to evolve a ligase, an RNA enzyme that could put two other strands of RNA together to form a new molecule. It was a step in the right direction, but if they hoped to make

something that could drive a living cell, they would need a more powerful type of ribozyme known as a polymerase.

A polymerase is more versatile than a ligase because it assembles smaller pieces in their "raw" form, rather than simply joining two pre-built strands. The difference is similar to that of between building a chair from scratch versus assembling one from IKEA. It's easier to mess up the chair from scratch—and that's actually a good thing in this case. In RNA, mutations can lead more readily to evolution, a point that would become important once the system was good enough to copy a chain of nucleotides long enough so that it could copy itself.

They evolved the system further. Bartel moved on from Szostak's lab to start his own at the Whitehead Institute and MIT, but kept working toward building a self-replicating bit of RNA. He tweaked the ligase, using both in-vitro evolution and rational design, until he had an RNA polymerase, a ribozyme that can build a new RNA molecule one nucleotide at a time. Bartel's polymerase had, he said, "...the type of chemistry that you'd need early on in evolution." It could attach a single nucleotide to the end of a short chain of other nucleotides, and could continue attaching more. The molecule was a first, a human made bit of RNA that could piece together another bit of RNA.

The "Bartel Ribozyme", as it is known, contains a couple of hundred nucleotides but can at best copy short sequences of between ten and twenty, and even then only at a slow rate. "It needs to copy things that are ten to twenty times longer, a lot more efficiently," said Szostak, "I think eventually someone will figure out how to do that." In the meantime, the effort has given Bartel a renewed appreciation for the original transition from chemistry to biology.

"You really get an appreciation for how difficult it would have been for an early RNA-based life form to emerge when you start trying to make one," he said.

Szostak believes that an RNA-based enzyme still holds promise but that there might be easier and more enlightening ways to approach the problem of self-replicating information molecules. Dropping the focus on making an RNA replicase directly, he asked his researchers working on information molecules to look for "purely chemical" solutions to the problem of replication. Szostak's first successes with replicating membranes had been unnatural—vesicles forced through a tiny hole—but they helped lead to Zhu's more elegant process of accumulation and shearing. Perhaps a similar approach would work with RNA—try something that seems unnatural to gain insight into a better way toward the original goal of a robust ribozyme. Their information molecule might not have to have enzymatic properties right from the start. Perhaps it didn't even have to be made of RNA. With these thoughts, Szostak set his lab to explore new, wholly human-made chemistries.

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Whether manmade or natural, certain principles of information storage remain the same. RNA is composed of four essential building blocks, the nucleotides Adenine, Guanine, Cytosine, and Uracil. These *bases* pair with each

other according to the rules discovered by Watson, Crick, and Chargaff in the 1940s and 50s. Guanine pairs with Cytosine. Adenine bonds with Uracil. Thus an open, unfolded string of RNA will naturally serve as a template for the complements of each base to stick to and self-assemble. Instead of relying on an RNA molecule's shape to string nucleotides together, Ricardo's approach relies on this templating behavior.

An actual RNA molecule is then just a single strand of A's, G's, C's, and U's—unlike DNA's famous "double helix" structure. The bases are held in place by a sugar molecule, ribose, and a phosphate group attached to each A, G, C, and U. The sugar-phosphate bits stack up like vertebrae in a spinal column, allowing the molecule to flex and bend, while still holding together. Sometimes this chain can fold up to assume a complicated physical structure, the property that underlies Cech's discovery and that gave Szostak and Bartel the idea that an RNA enzyme might work as the basis of a synthetic life form. If, instead, RNA is to serve as a template for replication, it needs to stay relatively open, exposing its unpaired bases to the environment so that free-floating nucleotides can attach piece by piece, hopefully forming a mirrored copy of the original.

If there is an enzyme around, it will grab the right nucleotide and put it in the right place and the right orientation in the new RNA. Without enzymes, the reaction proceeds according to the laws of thermodynamics—free nucleotides drift until they happen to land on the right spot, and in the right orientation, to stick to the template. Compared to doing it with enzymes, this non-enzymatic approach is absurdly slow.

Alonso Ricardo is another member of the Szostak lab working on information molecules. A post-doc in his late twenties, maybe early thirties, he speaks with a friendly Columbian accent. He and his colleagues are looking for ways to get information molecules to reproduce in the absence of enzymes.

"If you're in a laboratory and you're trying to model a Darwinian system in a membrane, it's too slow," Ricardo explained. "So what we do is, make changes, chemical changes that allow us to study the system on a more reasonable timeframe." Otherwise, he continued, he'd have to wait weeks for anything to interesting to happen.

A typical RNA or DNA molecule, acting without enzymes, can add about one base per day. With even the simplest functional RNA molecules running into the hundreds of bases, this rate is not fast enough for practical research.

Enzymes, on the other hand, can increase the rate by billions. Another post-doc in the Szostak lab, Quentin Dufton, explained, "Any reaction that happens, that an enzyme catalyzes, will happen at some rate spontaneously. Enzymes just speed things up by sometimes a factor of ten to the ten." In monetary terms, that's the difference between having a dollar and being Bill Gates.

Dufton went on to explain, "There are reactions that in nature will only happen on this planet once a day—the whole planet—but if you've got an enzyme that catalyzes it and increases the rate by ten trillion times, then it

happens in every single cell in our body dozens of times or hundred of times a second."

Without enzymes, Ricardo needs different ways to speed up reactions. He makes chemical changes to the nucleotides to help them stick to a template faster. Because of the complexity of the molecules, however, making changes that speed up the reaction can lead to other unwelcome consequences. For example, Ricardo explained, "you don't want to make modifications that make at the end nucleic acids that are too stable…you actually want them at some point to come apart. You have to be careful not to stick them together so they don't come apart."

He explained that tinkering with the chemical structure of RNA is often a delicate balance. "Every time we make a change of something that is from the natural system, and we try it, then we discover that we have to make another change to compensate. In order to have a net gain, you have to have multiple changes."

To keep track of how his changes affect the replication of his system Ricardo sticks with short chains, usually templates of twenty to thirty bases. He also typically gives the replication a head start by including "primer" molecules, preassembled chains of RNA that bind with part of the template.

One of Ricardo's chemical tricks is to use "activated" nucleotides—bases that have a sort of power-boost by the addition of a couple phosphate ions. The extra energy allows the activated nucleotide to more readily bond with other nucleotides. With the primer in place, and by using high concentrations of activated nucleotides, Ricardo can study and manipulate the mechanism by which the primer grows or "extends."

Ricardo has other tricks to get faster, more accurate replication. For example, instead of ribose or deoxyribose—the sugars found in RNA and DNA respectively—Ricardo is working with threose, a simpler sugar molecule with different chemical properties.

Ribose is basically a ring of five carbons with oxygens and hydrogens attached. Deoxribose—the "D" in DNA—is the same thing, minus the oxygen. Threose is a four carbon ring; Ricardo believes that its less complicated structure will make the resultant molecule, TNA—for *threose-nucleic-acid*—more rigid.

Ricardo's latest concoction uses a nitrogen-based "amine" group to make the threose sugar even more reactive. This gives the system a speed boost, "Instead of one nucleotide per day, now were going at one nucleotide per hour," he explained. The added group gets reflected in the new molecule's name, *two-prime-amino-threose*.

Ricardo and a team of researchers are working on first synthesizing the new sugar and then seeing if has the reactive properties he hopes. This is the sort of detailed chemical experimentation that goes into deciphering life's secrets. Individual atoms in molecules can make huge differences. The work has none of the romanticism that might pop up in imagining the work of building life from scratch. Long hours at the bench, meticulous measuring, endless pipetting—just

as in the membrane work, much of the frontline assault falls to biology's cannon fodder: the grad students.

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Craig Blain is a first-year student at Harvard Medical School, on rotation through a number labs affiliated with Harvard medical research. Before coming to the Szostak lab, he did a rotation with Pam Silver doing synthetic biology, a related field that often gets confused with what Szostak and his crew are up to.

"Synthetic biology, there's more direct application with what you're doing," Blain said, "The lab I was working in, they're trying to engineer bacteria to produce Hydrogen as a biofuel. So there's kindof that immediate satisfaction to making something that I guess is commercially viable."

Synthetic biology as practiced by researchers like Pam Silver at Harvard or Drew Endy at MIT uses existing living systems, like yeast cells or *e. coli* bacteria, and reprogram them to do useful things that they wouldn't normally do, like create hydrogen. It's a different approach, akin to customizing a car, and just like car parts adhere to standards, synthetic biologists like Endy are creating a vast catalog of "standard" biological parts—strands of DNA that can be connected with other strands of DNA and then inserted into a cell to give it some new functionality. "The labwork is more straightforward," Blain explained. "It's becoming relatively easy to engineer these things."

The work in Szostak's lab is less practical, more fundamental, and more mysterious. They're trying to build life from the bottom up, starting with the laws of chemistry and physics. "It's a very different problem, this is more of a chemistry problem," Blain said. "It's not just about getting the right atom in the right place, it also has to be in the right orientation," explained Blain. "A really tricky thing in organic synthesis is to get those orientations right."

Because Blain is relatively new, he's working on the two-prime-amino-threose that Ricardo designed. Earlier in the day, Ricardo gave him a fist-sized round flask—the classic chemistry beaker shape—with a fine white crystals coating the inside. The crust is basically threose, with various temporary attachments that prevent some parts of it from reacting or provide energy to encourage other parts to react—like scaffolding on a nano-sized building.

Blain wants to remove part of the scaffolding to make room to attach the amino group, really just a nitrogen atom. To do so, he has to take one invisible piece and attach it to another, slightly larger but still invisible piece. To the non-chemist, it's just a bunch of clear liquids and white powders.

First, Blain moves Ricardo's crystals to a smaller flask. To do this, he adds an organic solvent to dissolve the white residue, swishes it around, and then carefully pipettes the solution into a plum-sized beaker. With the solution transferred, he now must evaporate the solvent.

The evaporation process requires a rather large glass contraption with a two-liter-sized glass funnel suspended about three feet off of the bench, inside of a chemical hood. A two-foot network of glass tubes and chambers winds

diagonally down from the vicinity of the funnel. Blain pours a bucket of dry ice nuggets into the funnel; they look like steaming packaging peanuts. He tops them off with acetone—nail-polish remover, then attaches the small beaker containing threose precursor and solvent to the other end of the machine, lowers it into a bath of room-temperature water, and flips a switch to start the beaker rotating. The temperature difference between the water bath and the dry ice is supposed to evaporate the solvent quickly. It takes thirty minutes.

With the first solvent gone, the threose precursor turns back into dry crystals. One of the bits of molecular scaffolding attached the threose is a silicon group. It's blocking a particularly reactive site on the sugar, making sure that uninvited molecules don't bond where they're not wanted. Blain wants to remove the silicon group; to do so he needs to use fluoride ions. Before he can do that, however, he needs to add a new solvent.

This second solvent is extremely sensitive to water and thus must be kept under inert argon gas. Blain fills a rubber balloon with gas, attaches it to a needle, and punctures the sealed top of a brown glass bottle of solvent. With a two-inch needle attached to a syringe, he withdraws the amount he needs, the balloon of argon replacing the lost volume. He adds it to the threose precursor and then, with a different syringe, injects the beaker with the fluoride solution. He drops in a stir bar—a little pill-like piece of plastic coated metal that, in the presence of a rotating magnet will spin, stirring the mixture. "Last time," he says, "it turned slightly red."

The ordeal of moving the threose precursor to a smaller flask and then removing the protective silicon-based scaffold takes an entire afternoon. Completing the two-prime-amino-threose will take several such steps, adding and removing scaffolding, reacting, transferring. Once the sugar is ready, Ricardo will be able to attach it to nucleobases Adenine, Cytosine, Guanine, and Uracil to form nucleosides—the precursors of nucleo-*tides*—attach phosphate groups that activate the molecules, and then throw them into a beaker with a template to see how good of a replicating molecule they form. The entire process will take months.

If everything works as planned, Ricardo, Blain, and Szostak will soon have a TNA molecule that can replicate itself without the help of enzymes. If they succeed, it will be a coup. TNA has been found nowhere in nature; it's an entirely human creation. Getting it to replicate would be a significant step toward building a working protocell, but even then, a significant hurdle still awaits. This replicating molecule would have to be able to evolve some sort of function, such as catalyzing the formation of more membrane material. Once the molecule does something, it can be pressured to do it better—this is a necessary condition for natural selection. Replication is a neat trick, but replication plus evolution is life. Predictably, Szostak's got someone on it.

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Two buildings over from the Harvard Museum of Natural History—a classic New England red brick edifice—the futuristic, glass-encased, Northwest Laboratories building stands at the top of a slight grassy incline. The building is brand new; only about half of its four floors are occupied. On the top floor, looking out on a vista of the tops of the buildings on the east side of the Harvard campus, Irene Chen and her group are trying to figure out how a chemical system might gain the capability for open-ended evolution.

When Chen worked in Szostak's lab at Massachusetts General Hospital, she studied vesicles and discovered that the presence of RNA inside would allow them to steal lipids from other vesicles and thus grow. Now, in her own lab at Harvard, she and her post-docs and a few grad students and undergrads study how replicating RNA behaves when it makes mistakes. Their goal is to understand how such errors constrain the amount of information—and complexity—that can be stored and passed on, resulting in evolution. The work, like much of the Szostak approach to protocells, applies equally to origins of life scenarios as it does to modern synthetic approaches. Life's origin on Earth is the only example researcher's like Chen have to study, so if they want to find out what other forms of life are possible, they have to start with trying to understand what already happened.

"I've always been a big fan of science fiction and thinking about aliens or whatever," Chen said. Behind her desk, a large cardboard cutout of Ian McKellen dressed as Gandalf from the Lord of the Rings movies watches over the scene. "Thinking about alternative life forms and what else is possible...some of this research allows us to think about 'what are the alternatives to life as we know it?' "

For life to have alternatives, it has to have the capacity to change, which means in part having the capacity for diversity. This is a problem that any genetic molecule will have to solve, be it DNA, RNA, TNA, or some other chemistry. Chen studies RNA at the moment, because it is the best understood. One significant problem is that in a system of genetic polymers left to compete with each other in a test tube, the population will shift toward the molecules that are the most reactive, or the ones that happened to be most abundant at the start. In such a situation, the most reactive molecule might be simply a chain of the most reactive nucleotide—a homogenous sequence of all A's, for example. Such a molecule contains almost no information and as such might "win", but have no capacity to evolve.

"The question is, how can you increase the complexity that's contained in the sequence?" Chen asked. Like Ricardo and Szostak, she's looking at non-enzymatic replication, where nucleotides will self-assemble on a template without the assistance of any complex helper molecules. "We know that sequence complexity has increased over time. How can you get to that situation from just simple mechanisms?"

To help answer this question, Chen and her group study how RNA behaves when it makes a mistake—incorporates a mutation—as the most obvious way for a molecule to gain complexity. Mutations drive change, but too

many will inhibit it, preventing a sequence from passing on enough useful information to the next generation. German theorist Manfred Eigen studied this phenomenon in the 1970s and developed an equation that, for a given mutation rate, gives an estimate on the length of the longest genetic polymer chain the system can replicate.

Eigen identified that what is important is that the relationship between the length of the sequence to be copied—a proxy for how much information it can contain—is shorter than the average number of bases a system can copy before it makes a mistake. For example, Chen explained, "If you have a sequence of ten, and you make a mistake every twenty, then you're pretty much okay because you can make an entire sequence without making a mistake. If you make a mistake every five, then you're in trouble."

In this crucial way, the error-rate determines the length of the genome, which in turn determines whether or not it can have any sort of function. Without function, there is no basis on which genetic polymers can compete, nothing that would give one molecule an advantage over another, thus no selection pressure and no evolution.

"People haven't been able to synthesize RNA oligomers long enough that can have lengths that are close to functional capabilities," said Sudha Rajamani, a post-doc in Chen's lab. Rajamani is a former protein chemist who grew up in India before working with David Deamer at UC Santa Cruz. One problem is that the error rates of current non-enzymatic replication setups cap the maximum genome at around twenty-eight nucleotides long. The smallest known natural RNA ligase—a molecule that can stitch two others together—is around fifty-one nucleotides long. Using current techniques, this seems to block the possibility of evolving a functional RNA using a purely non-enzymatic approach. The so-called *error-catastrophe* occurs before such a molecule is halfway done replicating. Rajamani has discovered something, however, that makes her think this problem will soon be solved.

She thinks that a previously poorly understood process, known as *stalling*, might push back the error-catastrophe enough to create longer chains. Stalling occurs when a primer, growing against a template, incorporates a nucleotide not called for by the template—a mismatch, in other words. This has the effect of slowing down the addition of subsequent nucleotides. Rajamani recently found that this slowing down effect gives a population of primers enough time to separate from their templates and re-attach to other templates, some of which will call for the nucleotide that in the previous template was a mistake. Thus, what was an error is no longer an error, and the error-rate for the population of molecules as a whole goes down, which in turn increases the maximum length of a genome predicted by the Eigen equation.

Plugging in the new numbers, Rajamani can get close to one hundred nucleotides as the theoretical length of a genome that could replicate without enzymes. She explained that once you can get chains over fifty, "the rest of the scenarios are much more feasible."

With a critical number of nucleotides that can assemble and replicate without the help of enzymes, RNA chains with some function become possible. Non-enzymatic replication may in this way lead to building the robust ribozymes that have so far eluded Szostak and Bartel, but Rajamani's work is still in preliminary stages—other labs will have to take an independent look. She's optimistic, however, "This is the first step toward evolving a ribozyme, which can evolve a ligase, which can evolve a polymerase, and then it's life."

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Rajamani's optimism is not unusual, but the life problem is a complex beast. Not only are each of the components—membrane and genetics—difficult problems on their own, integrating them to make a whole system adds a layer of complexity. Szostak has replicating membranes, and he thinks that he'll soon have a replicating information molecule. His lab and others have even put bits of replicating DNA, using pre-existing enzymes, inside of vesicles and, by using the extrusion method, and had the system divide and "reproduce." But until the information molecule actually does something for the system, the components remain separate, not parts of a greater whole.

"When the RNA can qualitatively affect the membrane", explained Quentin Dufton, Szostak's post-doc and membrane expert, "you can call them coupled, not just two things that happen to be in the same place."

"The actual restriction at this point isn't so much getting the RNA inside and then growing and dividing the vesicles, it's having the RNA that's inside do something," said Dufton. "You can have genetic material inside, but if it doesn't cause any change in the vesicle, the container, or in itself, it doesn't matter. It's not really genetic information."

Dufton and others would like to see a way of marrying some sort of RNA activity to the vesicle. The best thing they have so far, he said, are RNAs that can catalyze the formation of more lipids, the building blocks required to make more membrane . Dufton would like to see RNA that catalyzes lipids that are chemically different from the membrane, giving it different properties.

"There's no reason why there couldn't be a ribozyme that could catalyze the addition of a phosphate to a carbon in a fatty acid," he said. Such a change would alter the chemical composition of the vesicle, perhaps making it stronger. The effect wouldn't have to be large he said, when the new lipids reached ten percent, they would start to have a real effect on the vesicle.

"If you had a scenario where you had vesicles made of fatty acid in a soup of stuff, but they were very unstable, and then for some reason there was an RNA which catalyzes the addition of glycerol to the fatty acid, that vesicle in the solution would all of a sudden have a huge selective advantage. Even if it's only a few percent of the molecules that change, if it changes the properties of the membrane, all of a sudden, that vesicle doesn't fall apart, and if it's growing and dividing..." the others will die off and the population shifts to favor the new, stronger membrane versions.

This of course requires genetic material that both affects the membrane and is able to make copies of itself. Both of these properties, combined with a membrane that can grow and divide, open up the potential for the system to evolve. "That's pretty close to being alive," Dufton said.

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Containment, replication, and evolution: by breaking up the life problem into simpler pieces, Szostak and his colleagues have made incremental progress toward building a purely synthetic protocell. "It was really clever of Jack and everybody to strip it down to the simple essentials," said Irene Chen. If his approach works, Szostak's creature could both shed light on the origin of life on Earth and provide a foundation for a whole new category of what philosopher Mark Bedau calls "living technology." The challenges left are still daunting and the span from nonliving to living matter remains unbridged, but reduced to a series of smaller challenges, Szostak and his colleagues believe that the life problem is solvable.

Some in the protocell community, however, question whether solving the life problem piece-by-piece will work. David Deamer has been working on both genetic and membrane issues relating to origins of life for over forty years. Now a professor emeritus at UC Santa Cruz, he has time to reflect on the field and try experiments that others might regard as "crazy." In particular, he's interested in areas around volcanoes and sea-vents.

"We actually go to a natural environment that we think is a model analog of the early Earth, and we try to run some of these things we do with a high-tech laboratory with ultra-clean conditions," said Deamer. In recent years he's traveled to the wilds of Kamchatka, in Russia, and the volcanic islands of Hawaii, looking for pools of warm water that might replicate conditions on the early Earth.

"We see how robust those things are in a hot volcanic environment like we think where the origin of life was." He's skeptical that the simple conditions of a test-tube can provide enough complexity to evolve a truly effective information molecule or containment structure. "I'm looking for robustness of chemical systems beyond the laboratory," he said.

Referring to the attitude that many in the field hold toward his recent work he said, "These guys are laboratory guys and they rebel against this kind of stuff because they say 'oh its so crazy to go into such an uncontrolled environment.'" Deamer says that this is because scientists are trained to be strict reductionists, to "be as clean as possible and get everything perfect and…they're not allowing things to occur in a way that is enlightening." He calls this problem *insufficient complexity*.

"That's why the ribozyme didn't work…they tried to get something to emerge by selective evolution, in vitro evolution, but they got limited because it was insufficiently complex in some sense." Deamer thinks that the problem is solvable, but that there are an overwhelming number of possibilities to try. "One laboratory, a couple laboratories at most, have explored one tiny fraction of a

near infinite space," he said, in reference the vast number of possible chemistries and genetic sequences to explore. "The early Earth had half a billion years to explore that same space in a global experiment of combinatorial chemistry, trying all these things out."

Deamer respects that the traditional method of reductionist science: isolating variables, understanding parts of a problem, trying one thing at a time, is absolutely crucial. He wonders, however, if by being so strict, scientists miss chances to capitalize on serendipity. "Reductionism—wonderful—it carries you a tremendous distance in understanding things. But going the other way is also very illuminating. In keeping it simple, perhaps we are missing one or more ingredients that would otherwise allow a recognizable living system to emerge."

As an example of the other direction, he points to Stanley Miller's 1952 experiment in which he attempted to recreate conditions on the primordial Earth by combining simple molecules—water, methane, ammonia, and hydrogen—into one container without a clear idea of what might emerge. Miller found that he was able to create many of the more complex building blocks for life with this method. "I think that Miller showed us how to go in the other direction, where you expect *something* to happen, even though you don't know what it's going to do," Deamer said. He continued on to point out that if Miller had left one chemical, ammonia, out of his experiment, his system would have been insufficiently complex, and no amino acids would have been produced.

Deamer thinks a similar approach will be required to ultimately assemble a chemical system that might qualify as "alive." Speaking of coordinating the various molecules required for containment, replication, function, and evolution, he said, "If you don't have it all together; if one of those things is missing, it's not going to be alive. So it can't be one thing first, then another, in sort of a hierarchy. It's got to be coevolution of all these parts."

He thinks doing this will require a new mindset in the field. "We're just trained as scientists to be reductionistic...my guess is it's more likely that we will find the way through a Miller-type boldness, where you simply do the experiment in a sufficiently complex environment and something is going to emerge that we will not have predicted."

Despite his calls for a new boldness, Deamer is careful not to dismiss the work of Szostak and others. He thinks that such incremental, compartmentalized understanding is crucial for knowing the components that need to be in play. Of Szostak, Deamer said "He's saying, 'let's just take chemical principles, and put biology aside, and let's see if we can just make this chemically'...and that of course is tall order because you're learning from scratch. But, you know, he might get lucky."

Though they differ on how it might happen, both Deamer and Szostak think that the creation of wholly novel organism from scratch is on the horizon. Recently published findings, such a Zhu's discovery of natural vesicle growth and division, keep Szostak optimistic that his approach may indeed go the distance. In August of 2008, Szostak and another protégé, Denver University researcher Sheref Mansy, announced that they had successfully performed elementary DNA

replication inside of a vesicle where the component nucleotides—the raw materials—were able to pass into the barrier while the DNA inside could not escape. This showed for the first time that an information molecule inside of a protocell could get the materials it needs for replication while maintaining the integrity of the whole system. In other words, the thing can eat.

Later, in the fall of 2008, Szostak and Mansy produced another paper showing that using heat to allow replicating DNA to "denature" or separate its strands so that they could replicate, would not destroy the membrane. Szostak pointed to this as an example of how his approach does in fact allow for surprises. "When we were thinking about this problem at the beginning, we thought 'well yeah heating them up that sounds good but if we do that to our membranes, they're just going to fall apart'. But it turns out, when we started doing experiments to determine if that's really true—it's not. The membranes are perfectly fine; you can boil them, no problem, the DNA or RNA doesn't leak out, they stay structurally intact."

"What we thought was potentially a huge problem," Szostak said, "turned out to be really not a problem. Now I don't know if that's going to happen with every potential problem, but it's kinda nice when some things turn out to be a lot simpler than you initially expected." He's cautious however, "You never know if one of those little nagging problems might all of a sudden blow up into a brick wall."

Ultimately, Szostak hopes that building a synthetic protocell will shed light on how life evolved on Earth, even if his particular design isn't plausible in a pre-biotic environment. For the time being, a human-designed living thing, built from scratch, remains a goal unattained. Nearly a decade on from his roadmap paper, Szostak is one of a few people on the planet who intimately understands the full scale of the challenge. He is undeterred. "I've always found that, when you're trying to build something that's new, it seems very difficult at the beginning," he said. "When you start, maybe you can't even imagine how you would build something that you have in mind. But, you know, gradually you get there sort of step-by-step and eventually you find one way to do it. But by that time, you usually have two or three other ideas of how it might work. Almost inevitably it turns out that there are two or three or *lots* of ways of doing something that initially seemed almost impossible."

Sources:

In-Person Interviews:

Alonso Ricardo, Post-Doc at the Szostak Lab, interviewed multiple times at Szostak's Lab at Massachusetts General Hospital, Fall 2008->Spring 2009

Craig Blain, Grad Student in the Szostak Lab, interviewed multiple times at Szostak's Lab at Massachusetts General Hospital, January 2009

David Bartel, Head of the Bartel Lab, interviewed in his office at the Whitehead Institute, MIT, March 2009

David Deamer, Professor Emeritus, UC Santa Cruz, interviewed at the 2009 AAAS conference in Chicago, February 2009

Irene Chen, Head of the Chen Lab, interviewed in her office at Harvard, October 2008

Jack Szostak, Head of the Szostak Lab, interviewed in his office at Massachusetts General Hospital, January 2009

Mark Bedau, Professor of Philosophy at Reed College, interviewed at Starbucks in Portland, OR, January 2009

Quentin Dufton, Post-Doc at the Szostak Lab, interviewed at Szostak's Lab at Massachusetts General Hospital Massachusetts General Hospital, October 2008

Rafael Bruckner, Grad Student in the Szostak Lab, interviewed at Szostak's Lab at Massachusetts General Hospital, January 2009

Sudha Rajamani, Post-Doc at the Chen Lab, interviewed multiple times at Chen's Lab at Harvard, Fall 2008->Spring 2009

Phone Interviews:

David Krakauer, Santa Fe Institute, October 2008
Harold Morowitz, George Mason University, November 2008
Sheref Mansy, Denver University, March 2009
Tracey Lincoln, Scripps Research Institute, March 2009

Books:

Bedau, Mark. Artificial life VII: Proceedings of the Seventh International Conference on Artificial Life. Cambridge, Mass.: MIT Press, c2000.

Bock, Gregory., Goode, Jamie. The Limits of Reductionism in Biology. Chichester; New York: J. Wiley, 1998.

Dawkins, Richard. The Ancestor's Tale: A Pilgrimage to the Dawn of Life. London: Weidenfeld & Nicolson, 2004.

Gesteland, Raymond F., Cech, Thomas R., Atkins, John F. The RNA World: The Nature of Modern RNA Suggests a Prebiotic RNA World. Cold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory Press, c1999.

Levy, Steven. Artificial Life: The Quest for a New Creation. New York: Pantheon Books, c1992.

Morange, Michel. Life Explained. New Haven: Yale University Press; Paris: Éditions Odile Jacob, c2008.

Rasmussen, Steen., et al. Protocells: Bridging Nonliving and Living Matter. Cambridge, Mass.: MIT Press, c2009.

Articles:

Cech, Thomas R. "Exploring the New RNA World." www.nobelprize.org. 3 December 2004.

Cech, Thomas R., Zaug, Arthur J., Grabowski, Paula J. "*In Vitro* Splicing of the Ribosomal RNA Precursor of Tetrahymena: Involvement of a Guanosine Nucleotide in the Excision of the Intervening Sequence." Cell Dec. 1981: 487-496.

Deamer, David. "First Life and Next Life." Technology Review May/June 2009.

Eisen, Jonathan A. et al. "Macronuclear Genome Sequence of the Ciliate Tetrahymena thermophilia, a Model Eukaryote." PloS Biology Sept. 2006: e286.

Gilbert, Walter. "The RNA World." Nature 20 Feb. 1986: 618.

Johnston, Wendy K., Unrau, Peter J., Lawrence, Michael S., Glasner, Margaret E., Bartel, David P. "RNA-Catalyzed RNA Polymerizatino: Accurate and General RNA-Templated Primer Extension." Science 18 May 2001: 1319-1325.

Kruger, Kelly., Grabowski, Paula J., Zaug, Arthur J., Sands, Julie., Gottschling, Daniel E., Cech, Thomas R. "Self-Splicing RNA: Autoexcision and Autocyclization of the Ribosomal RNA Intervening Sequence of Tetrahymena." Cell Nov. 1982: 147-157.

Szostak, Jack W., Bartel, David P., Luisi, P. Luigi. "Synthesizing Life." Nature 18 Jan. 2001: 387-390.

Zaher, Hani S., Unrau, Peter J. "Selection of an Improved RNA Polymerase Ribozyme with Superior Extension and Fidelity." RNA 13 2007: 1017-1026.

Zhu, Ting F., Szotak, Jack W. "Couple Growth and Division of Model Protocell Membranes." Journal of American Chemical Society 26 March 2009: 5705-5713.

Zimmer, Carl. "What Came Before DNA?" Discover 26 June 2004.