

A World Apart

A group of scientists with mammoth imaginations and the best biotech tools is piecing together a view of a prehistoric world where RNA ruled. This seemingly esoteric pursuit is generating modern-day approaches to fighting disease. >>

By Robert
Kuska



J.W. STEWART

» Without a time machine,

biologists may never know for sure what created the first organisms roughly four billion years ago. Did life on Earth evolve, as some suspect, from self-replicating RNA molecules? In the hypothetical “RNA World,” a vision of the primordial Earth, precursors of modern RNA were responsible for storing genetic information and catalyzing biochemical reactions—functions primarily associated with DNA and enzymes.

While the theory is controversial, its implications transcend academic debate. In recent years, discoveries about the potential capabilities of primitive RNA have generated a wealth of new information about the structure, biochemistry and biological diversity of modern RNA. That, in turn, has served as the intellectual spark for a new and rapidly evolving species of the biotechnology world—companies that hope to develop and commercialize cutting-edge, RNA-based diagnostics and therapies for a range of human diseases (see page 18).

“I don’t think that we are ever going to prove evolutionary origins from work on the RNA World because it’s just too hard to do,” says Jennifer A. Doudna, an HHMI investigator at Yale University, who publishes frequently on RNA. “But I think that we can get some very interesting clues from these studies, and it makes the work exciting.”

Although the term was coined in 1986 by Nobel laureate Walter Gilbert at Harvard University, the idea behind the RNA World first appeared in the scientific literature in the 1960s. At the time, several theorists were bouncing around the relatively new concepts of codons and complementary base pairing in an effort to explain the relationship between DNA and proteins. Among the issues they raised was the ultimate chicken-and-egg question: Did proteins evolve before DNA, or vice versa? DNA, after all, requires enzymes to replicate, but enzymes are themselves proteins whose generation depends on “instructions” contained in DNA code.

The central dogma that emerged in molecular biology within a few years of Watson and Crick’s epochal description of the double helix in 1953 is that DNA leads to RNA, and RNA leads to protein. That is, sequence information from DNA in the chromosomes of all eukaryotic cells is copied to messenger RNA (mRNA) in a process called transcription; mRNA, in turn, carries its information to structures called ribosomes (see *Bulletin*, January 2001). Within these tiny “factories,” mRNA’s information is translated in the manufacture of proteins—the fundamental building blocks of cells, tissues and organs.

Elegant though this description is, it still leaves the chicken-egg problem. How could the complex DNA molecule have come into existence in the absence of enzymes? In 1968, Francis Crick, the legendary codiscoverer of the genetic code, offered a tentative explanation. Noting that the ribosome is composed largely of RNA, Crick proposed that RNA could have preceded both DNA and protein as the source of life. If so, in its earliest, most primitive state, RNA must have possessed both the capacity to store genetic information like DNA and the catalytic abil-

ity of an enzyme to power a rudimentary cell.

Though the idea sounded attractive, Crick and his colleagues faced a monstrous task in proving it. It was like strapping artificial wings on a horse and ordering it to fly like Pegasus—every molecular biologist knew that modern RNA was a messenger molecule, not an enzyme.

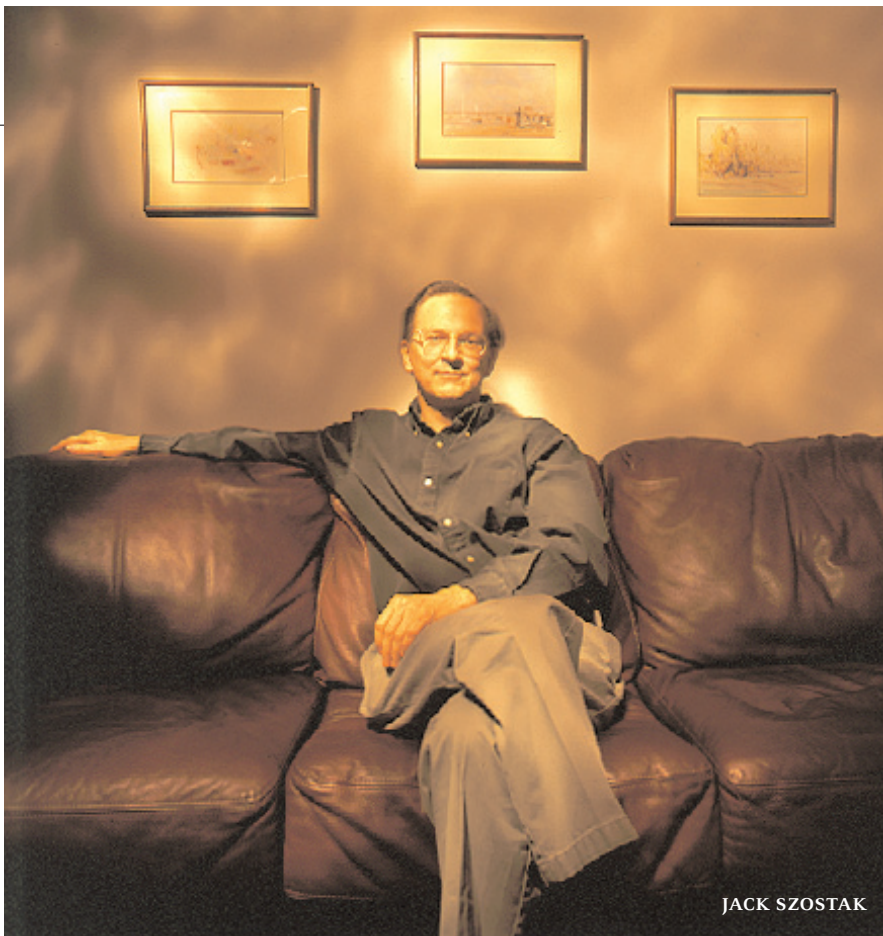
“We are really in the early days of RNA work, learning how RNA molecules fold, function and catalyze chemical reactions.”

—JENNIFER DOUDNA

JENNIFER DOUDNA



HAROLD SHAPIRO



JACK SZOSTAK

MARK WILSON

In the early 1980s, however, the horse sprouted wings. That's when two laboratories—one headed by current HHMI president Thomas R. Cech, the other by Sidney Altman at Yale University—independently and quite serendipitously discovered two distinct RNAs in the model organisms *Tetrahymena thermophila* and *Escherichia coli*. Under certain conditions, these RNAs were able to promote very precise cutting and joining of chemical bonds, thus acting like enzymes. In the often rigid, paradigm-driven world of biology, it was as unexpected as stumbling upon penguins nesting in the Florida Everglades. Jaws dropped.

The Nobel Prize-winning discovery of catalytic RNAs—now known as ribozymes—not only complicated the “DNA-RNA-protein” dogma, it added support to Crick's once tentative speculation. If mod-

ern living cells still lugged around fossils from an ancient RNA past, scientists might be able to dig them out and, in theory, develop a powerful model to explore the rudiments of primordial chemistry; Darwinian evolution; and, by inference, the biochemistry of modern RNA.

ARCANE VENTURES

Despite its obvious intellectual appeal, the RNA World was for the most part a quirky conceptual pastime in the years following the discovery of ribozymes. Though important new work dotted scientific journals, scientists unearthed only a half-dozen types of ribozymes that occurred naturally. This led many to believe that RNA World experiments had no practical applications.

“Ribozyme research was considered to be a very esoteric, ivory-tower sort of topic,” remembers Cech. “No one really dreamt of there being any commercialization or any practical spin-offs from the field at all.”

By the late 1980s, however, the theory of the RNA World began to attract the attention of researchers in biotechnology. As Cech observes, the field has never been the same since. “I went on Medline [the Internet database] a few weeks ago and saw that there were more than 2,000 articles that use the word ribozyme,” he says. “This is just a word that we invented in Colorado in 1981 for a lone example of a catalytic RNA. It has taken a worldwide effort to find other natural ribozymes [seven distinct structural classes and

more than 1,800 total examples have been described] and then to learn how to make unnatural ones for a full-fledged field to develop.”

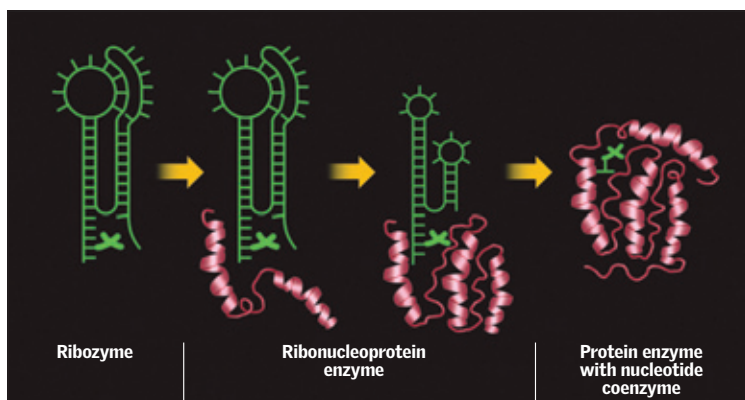
One of those leading the charge is HHMI investigator Jack W. Szostak, at Massachusetts General Hospital. Several years ago, Szostak and others believed the time was right to attempt to create ribozymes artificially by using a laboratory technique called *in vitro* selection. Szostak and colleagues begin their experiments with a test tube containing trillions of random-sequence RNA molecules. They screen this vast array of RNAs for some predetermined function, such as the ability to catalyze a specific chemical reaction or bind a target molecule. Those that don't make the grade are filtered out.

“Then we amplify the surviving molecules,” Szostak explains. At that point, the process is repeated, again and again. “The fact that this is an iterative process means that, in principle and actually in practice, you can start with a thousand trillion molecules in a little tiny tube. And you can find the one molecule in that tube that does a particular job.”

In short, Szostak and his colleagues crank up the forces of Darwinian selection to warp speed and then pull out the winner. “So many new ribozymes have been evolved from *in vitro* selection,” he says. “The range of chemistries these artificially produced ribozymes can catalyze has been greatly expanded. Yet we haven't observed this activity in living cells. It raises the interesting possibility that in an earlier era, ribozymes might have played a wider role than they do today.”

Like all laboratory techniques, *in vitro* selection is only a means to an end. For Szostak and others, the endpoint is not, as is sometimes reported, a bold attempt to recreate the RNA World, an epoch 50 times

“No one really dreamt of there being any commercialization or any practical spin-offs from ribozyme research at all.” —TOM CECH



The proposed transition from the RNA World, where ribozymes performed all the catalysis needed for life. Later, RNA worked in concert with random chains of amino acids. When RNA evolved to synthesize specific proteins (as in today's ribosome), the role of proteins grew. In the modern world, catalysis is mostly performed by proteins (some still with RNA cofactors).

ADAPTED FROM A CONCEPT DEVELOPED BY HAROLD B. WHITE III

itive RNA; indeed, he says, “there probably never were any genetic fossils. The last common ancestor of life on this planet, it seems clear, was a pretty complicated organism. It had 300 protein genes—ballpark. It had ribosomal RNA; it had a full set of transfer RNA [which carried the appropriate amino acids to the ribosome to build new proteins]. Whoever the last common ancestor was, he and his contemporaries more or less ate the guys who were still in the soup. We don't have them to compare to anymore.”

Why, then, this venture into the unknown? Why try to concoct a primitive RNA cocktail in a small glass tube? The answer is twofold. First, by performing in vitro selection experiments, scientists can learn to distinguish the biochemically possible from the impossible. As many point out, this nuts-and-bolts approach has direct applications in learning how to tweak the biochemistry of modern RNA and leverage its biochemical secrets to create new technologies to fight disease. “It is important to remember that we are really in the early days of RNA work, learning how RNA molecules fold, function and catalyze chemical reactions,” says Doudna, noting that such fundamental work is essential to develop effective RNA-based tools in medicine.

Second, the hypothetical RNA World serves as a unique model to study Darwinian evolution. Szostak would like to create a crude, RNA-based artificial cell that is capable of rudimentary metabolism. This would provide the first window to observe in real time the forces of Darwinian selection at work on primitive biomolecules. Through this window researchers could gain insight into key biological concepts

more ancient than the dinosaur era. The goal is to create a hypothetical model of a protobiotic Earth in which catalytic RNA might have evolved.

“It's all indirect in the sense that we really don't have an RNA World to study,” Szostak says. “We just don't know the conditions that would have been relevant at that time. Basically, it's all possibility.”

Sean R. Eddy, an HHMI investigator at Washington University School of Medicine in St. Louis, points out that there is no fossil record of prim-

From the RNA World to the Real World

More than a half-dozen biotechnology companies have been launched to develop and commercialize ribozyme-based diagnostics and therapeutics. The pioneer was Ribozyme Pharmaceuticals, Inc. (RPI), of Boulder, Colorado. Founded in 1992 by current HHMI President Thomas R. Cech, the company was organized to pursue the discovery from Cech's laboratory that some types of RNA, called ribozymes, can cleave other RNA molecules. RPI is developing therapeutic ribozymes to target the messenger RNA (mRNA) of proteins implicated in specific diseases. Like all proteins, disease-related proteins are encoded by genes, whose instructions are carried to the ribosome

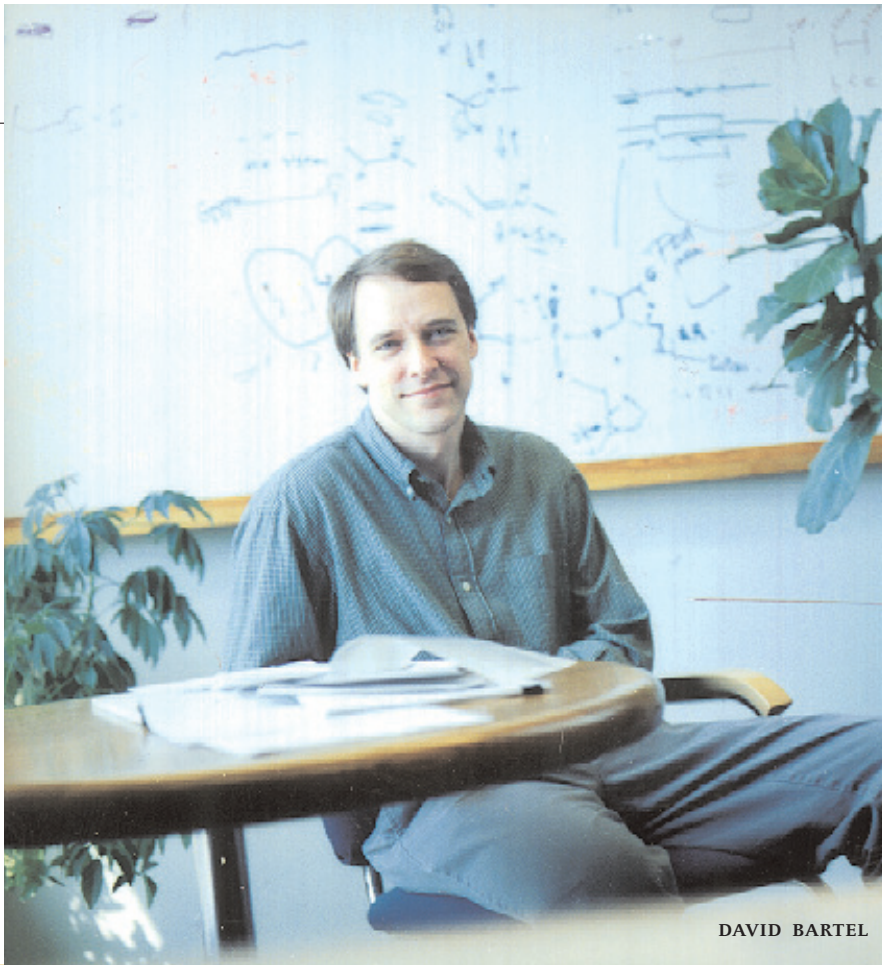
by mRNA. By synthesizing ribozymes that bind and cleave these mRNAs, RPI scientists are trying to prevent undesirable proteins from being produced.

After a decade of work, RPI has three ribozymes in early phase clinical studies to treat hepatitis C and cancers of the breast and colon. Another ribozyme is in clinical development to treat hepatitis B. Other companies that see promise in ribozyme-based therapeutics include Immusol, Inc., of San Diego; RiboTargets, of Cambridge, U.K. and Rib-X Pharmaceuticals, of New Haven, Connecticut, whose founders include HHMI investigator Thomas A. Steitz at Yale University.

Immusol uses ribozymes to “identify novel targets against which small-molecule-, antibody- or ribozyme-based drugs can be developed. Armed with a detailed knowledge of the structure of the ribosome and with techniques to screen large numbers of molecules at once, companies like RiboTargets and Rib-X are looking for ribozymes or other small molecules that will target the ribosome of a harmful bac-

terium and shut it down. In particular, they are targeting these potential antibiotic drugs to specific regions of the ribosome that don't seem to be susceptible to mutation. This means that the bacteria will be less likely to develop resistance to the drugs. RiboTargets is one of several firms also exploring the feasibility of using ribozymes to prevent or interrupt the process of replication in HIV, the virus that causes AIDS.

Still other young companies see ribozymes as the basis of new diagnostic tools. Archemix of Cambridge, Massachusetts, has licensed a technology developed at RPI to design a series of ribozymes whose activity is switched on or off by the presence of specific molecular targets. Called RiboReporters, these special ribozymes act as extremely sensitive biosensors. When they bind to target molecules, energy is generated that is detectable on various assays. According to Archemix, RiboReporters can be used to detect numerous molecules, ranging from ions to small molecules to proteins. —RK



DAVID BARTEL

KATHLEEN DOOHER

such as replication, translation, cellularization and metabolism.

Using in vitro selection, Szostak and other RNA Worlders have made tremendous progress toward this goal. Last year, David P. Bartel's laboratory at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology produced a ribozyme that was capable of generating a complementary strand of other RNA sequences. Though Bartel says that the fidelity and length of the copy needs to be improved, the work nevertheless established that RNA can catalyze its own replication, a capability consistent with the RNA World hypothesis.

If in vitro selection is one vast chemical lottery, then the jackpot belongs to the laboratory that creates the first so-called replicase. A replicase is a still-theoretical ribozyme that would have catalyzed the replication of RNA molecules, including itself, in an RNA World.

“Nothing has led to thinking hard about ‘origins’ more than the RNA World hypothesis.” —ANDY ELLINGTON

Though a replicase has eluded scientists for years, most in the field say that its synthesis is only a matter of time. “It would be a very nice achievement, for sure, but it would not be completely shocking because we know it is possible to get an RNA to do the kind of chemistry that is necessary for a replicase,” says Doudna.

“It seems to me that the next step is getting the process to work, to copy long templates with better fidelity and to make molecules that are in fact functional,” she continues. “If you really are making a replicase, then you are making a molecule that is able to make a copy of itself. And that would be a remarkable achievement. If that were done, one could certainly imagine setting up a system in the laboratory to actually watch the molecule evolve. That would be pretty exciting.”

Bartel, whose laboratory seems to be the front-runner in the search for a replicase, agrees the payoff will be witnessing the forces of evolution leap into action. “If the replicase is just replicating any RNA that is in a solution, then there won’t be any selective advantage for it to replicate faster or more accurately,” he says. “It will just be replicating all of the RNA equally. But, if it preferentially replicates its relatives, then you get an evolutionary line going that takes over. That’s when the fun begins.”

TIME WASTED?

There remain many skeptics. Some doubt that pre-cellular RNA-based life would have had sufficient time to evolve into the most primitive bacterial cells that fossil evidence has revealed. The earliest date to about 3.8 billion years ago, leaving a fairly narrow 150- to 500-million-year span between the end of Earth’s bombardment by solar system fragments and the appearance of the first simple bacteria. Therefore, some scientists, including Francis Crick, have indicated a preference for a theory called Panspermia, which speculates that earthly life had an extraterrestrial source. Others have explored the possibility that humble crystals of terrestrial clay might have served as “scaffolds” upon which the first genomes assembled themselves.

If either of these theories or some other were to be proven true, would studies of the RNA World have been a waste of time—a scientific dead end?

“Oh, good Lord, no,” says Andy Ellington, a scientist at the University of Texas at Austin, who has studied the RNA World and recently joined forces with a colleague to start a biotech company that he describes as an indirect spin-off of the RNA World. In Ellington’s view, it’s something like the advances in miniaturization and integrated circuit development that were made as a result of the space program. They’ve profoundly changed the way we live, even though “we don’t go to the Moon anymore.”

“Nothing has led to thinking hard about ‘origins’ more than the RNA World hypothesis,” Ellington says. “Even if NASA scientists were to break the news that life began with cosmic ‘seeds,’ the ideas that have emerged as a result of the theory would not be irrelevant, just displaced to the next step in our history.” **■**