FUTURE OF MEDICINE 2014

THE RNA REVOLUTION

Long overlooked as a mere cellular housekeeper, RNA has emerged as a path to **a new world of medical treatment**

By Christine Gorman and Dina Fine Maron

Illustrations by Anna Parini

Starting with the double-helical structure of DNA in 1953, the story of molecular biology has featured more characters than a Russian novel. Biologists have identified tens of thousands of molecules that direct and shape the organized chaos within the body's cells, and they have exploited those findings with thousands of drugs and treatments.

For decades the stars of the drama came from two camps: DNA, or deoxyribonucleic acid, which acts as a near permanent repository of genetic information, and proteins, which do the genes' handiwork. Protein discoveries have led to such medical advances as synthetic insulin, interferon and next-generation anticancer drugs. And gene therapy, using modified bits of DNA, has made headway against hemophilia, hereditary blindness and other previously intractable diseases.

Overlooked in this march of medical progress was a third type of biomolecule: RNA, or ribonucleic acid. Like its more famous sister, RNA contains genetic information, but it is less chemically stable than DNA and is often degraded by enzymes in the turbulent environment of the cytoplasm.

Although scientists have long known that RNA is intricately involved at some point in almost every cellular process, for most of the biomedical revolution they assigned it a supporting role, in the shadow of DNA and proteins. In the 1950s and 1960s biologists thought of RNA as a kind of Cinderella molecule, ferrying messages, coordinating supplies and generally keeping cells tidy. For decades this view stuck.

But that was before a few fairy godmothers (and godfathers) gave RNA a stunning makeover. A series of discoveries in the late 20th century revealed new forms of RNA that were nothing like humble housekeepers. On the contrary, these RNA molecules exerted an astonishing degree of control over the behavior of DNA and proteins—targeting specific molecules to increase or decrease their activity. By manipulating this RNA, scientists could potentially develop new treatments for cancer, infectious diseases and a wide range of chronic illnesses.

In the past decade or so investigators have raced to exploit this insight. The pace of discovery has accelerated, dozens of start-ups have formed to capitalize on new findings and now some promising treatments are in the offing.

Meanwhile an early trickle of financial interest has grown into a multibillion-dollar torrent. Among recent ventures, Editas Medicine received $43 million in venture capital for its launch at the end of 2013; the company is concentrating its

IN BRIEF

Three of the most important complex molecules in living organisms are DNA, RNA and protein. For decades biologists ascribed the most active roles in the cell to DNA and proteins; RNA was clearly important but rendered more supportive services.

A series of discoveries in the late 20th century revealed several previously unknown forms of RNA that play active, regulatory roles in the cells—determining which proteins are manufactured and in what amounts or even silencing some genes altogether.

These latest insights are allowing scientists to create a new world of experimental medications against bacteria, viruses, cancer and various chronic conditions that should work more effectively and precisely than many currently available drugs.

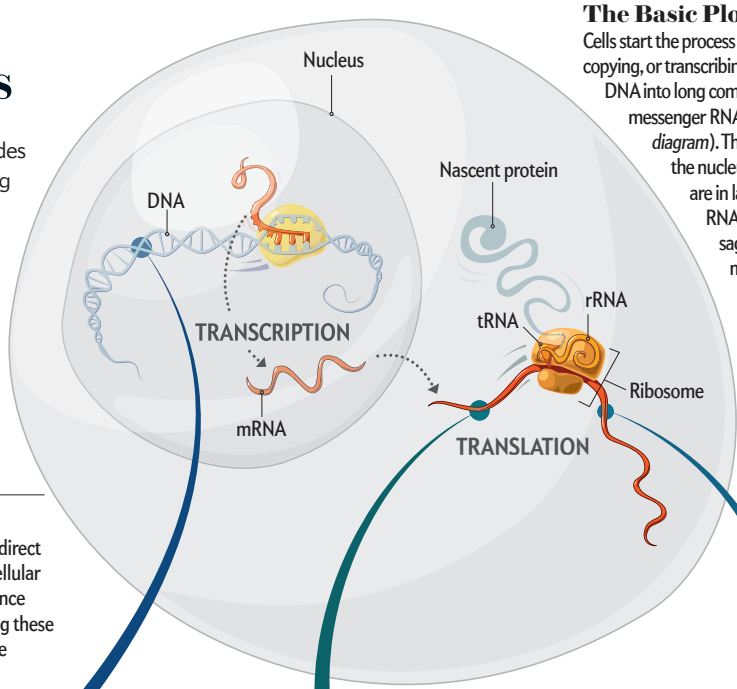
BREAKOUT PERFORMANCE

RNA Shines in New Roles

Scientists have known for decades about RNA's basic housekeeping duties in the cell. Research over the past few years, however, has uncovered new forms of RNA with surprising functions that could one day lead to more precisely targeted medical treatments.

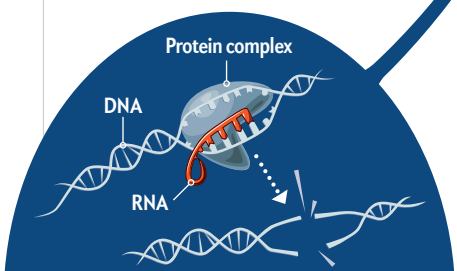
The Basic Plot

Cells start the process of manufacturing proteins by copying, or transcribing, the genetic code found in DNA into long complementary sequences of messenger RNA, or mRNA (*shown on left of diagram*). The mRNA then travels outside the nucleus, where ribosomes, which are in large part made of ribosomal RNA, or rRNA, translate the message into a growing protein molecule by linking specific amino acids together (*shown on right*). So-called transfer RNA, or tRNA, molecules find and slot the amino acids in place.

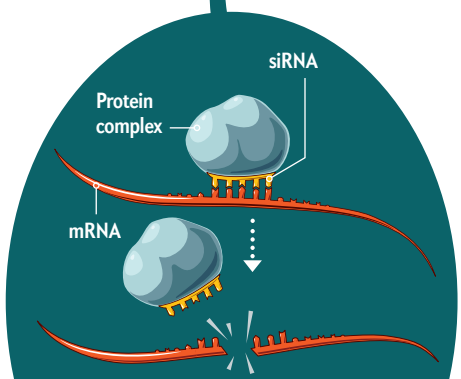


New Twists

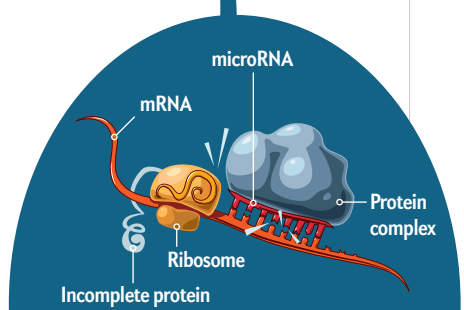
Recently discovered types of RNA can direct specialized proteins to block certain cellular processes from happening or even silence them entirely. Researchers are adapting these pathways to develop new, more precise medical treatments.



The so-called CRISPR system gained prominence as a genetic engineering tool in 2012. Scientists create a guide strand made up of RNA that complements the exact genetic sequence in the DNA that they want to modify. Then they attach the guide strand to a protein that cuts DNA in two. The combined RNA-protein complex searches out the targeted DNA sequence and permanently disrupts it. Small bits of corrective DNA can also be added at the same location, in a separate process.



Investigators create small interfering RNA (siRNA) molecules that complement the section of a messenger RNA they want to disrupt. The siRNA is then taken up by a complex of proteins that cut the singled-out mRNA at the spot indicated by the siRNA.



Researchers hope to manipulate microRNA, which gives cells the ability to change the production of specific proteins, to treat a range of diseases. Because the RNA of the microRNA does not have to be a perfect match for the mRNA whose translation is being affected, a small number of microRNAs can temporarily alter production of many different kinds of proteins.

efforts on the hottest new RNA technology, known as CRISPR. A slightly older company, Alnylam Pharmaceuticals, founded in 2002, received $700 million this past January to develop, among other things, its pipeline of RNA medications for devastating blood conditions, liver diseases and immune disorders.

The funding has come "in waves," says Robert MacLeod, vice president of oncology and exploratory discovery at Isis Pharmaceuticals, which has raised nearly $3.8 billion since it was founded in 1989. Its lead product, Kynamro, received approval from the U.S. Food and Drug Administration in 2013 as an RNA medicine for people with a rare genetic disorder that significantly interferes with their ability to process cholesterol, putting them at an exceptionally high risk of heart attack and stroke.

As with any rapidly expanding field, there have been a few bumps and detours along the way, and not every discovery will likely stand the test of time. Yet medical researchers are practically giddy with excitement—as if they had found a new continent to explore in search of potential breakthroughs.

SUPPORTING ROLE

IT IS EASY TO SEE WHY molecular biologists would assign starring roles to DNA or proteins rather than RNA. DNA's main subunits—adenine, thymine, cytosine and guanine, or A, T, C and G—constitute the basic instruction manual for growing just

Illustration by Josy Conklin

about every living thing on the planet. And one of the most important processes that DNA provides directions (or codes) for is the creation of proteins.

Proteins, for their part, give cells their three-dimensional structure and allow them to perform many jobs; they provide the skin's youthful spring and the heart's lifelong strength. They also turn DNA on and off in response to environmental cues, determine how well cells use sugar and regulate the ability of neurons to relay signals to one another in the brain. The vast majority of today's medicines—from aspirin to Zoloft—work by manipulating proteins, either by blocking their function or by altering the amount that is produced.

Just because most medications affect proteins, however, does not mean that investigators have been able to develop drugs that act on all the proteins they would like to target. The most common pharmaceutical remedies consist of small molecules that can survive being swallowed and passed through the acidic interior of the stomach. Once absorbed from the digestive system, they must fit into the active locations on their target proteins the way a key fits a lock. But there are certain groups of proteins for which this traditional approach will not work. The proteins bury their active sites too far inside narrow channels, or they do not even contain an active site because they make up part of the cell's internal skeleton, which renders them "undruggable," MacLeod says.

This roadblock is what the new RNA medicines are designed to overcome—though how they could do so has not been obvious until recently. As biologists have long known, RNA serves as a talented go-between, copying, or transcribing, DNA's instructions into a complementary sequence (matching a C for every G, for example) and then translating that code into three-dimensional proteins. So-called messenger RNA (mRNA), which is generated in the nucleus, travels to the cytoplasm, where structures called ribosomes and transfer RNA (tRNA) work together to read the message and connect amino acids (nitrogen-containing compounds) into long chains that become proteins. But RNA can do much more.

A NEW SHOT AGAINST HEPATITIS C

Targeting a microRNA in liver cells could disable a silent killer

By Christine Gorman

Twenty-five years ago no one had even heard of the hepatitis C virus. Today it is a leading cause of liver cancer and a major reason why people get liver transplants. Globally it kills about 350,000 people a year; in the U.S., more people now die of hepatitis C than of AIDS.

A STAR IS BORN

THE GROUNDWORK for RNA's breakout performance was laid in 1993, with the identification of the first microRNAs. These uncharacteristically short stretches of RNA attach themselves to strands of mRNA, preventing ribosomes from making any progress in assembling a protein [*see box on preceding page*]. Cells apparently use microRNAs to coordinate the production schedule of many proteins—particularly early in an organism's development. Five years later researchers made another breakthrough when they demonstrated that different short RNA molecules effectively silenced the translation of a gene into protein by cutting up mRNA. That landmark discovery later netted a Nobel Prize, in 2006.

By this point, everyone—not just RNA specialists—was seemingly interested in using the once overlooked molecule to influ-

The infection can be cured—albeit with debilitating side effects. Standard treatment with interferon and ribavirin causes fever, headaches, fatigue, depression and anemia. Such therapy may last as long as 11 months and clears the infection in 50 to 70 percent of cases. The recent addition of protease inhibitors, a class of medications that was first used against HIV, has improved cure rates and lessened treatment time. Unfortunately, the newer drugs work only against the type of hepatitis C most common in North America, Europe and Japan, so they are not equally effective around the world.

RNA medications may better that outlook. In 2013 researchers showed that targeting a particular microRNA in liver cells with an experimental drug called miravirsen dramatically decreased the amount of hepatitis C virus in most patients receiving treatment, in some cases to undetectable levels. The experimental medication consists of a short sequence of DNA whose "letters" are exactly complementary to the RNA letters found on the microRNA, allowing the drug to home in on its objective precisely.

The microRNA in question, known as miR-122, plays a key role in the production of many proteins in the liver. It seems to enhance their manufacture, however, rather than suppressing it as so many microRNAs do. Once the hepatitis C virus gains entry to a cell, it attaches itself to miR-122, ensuring that multiple viral copies are made. Blocking miR-122 ends up blocking virus replication as well.

The main side effect of miravirsen therapy was redness at the injection site, which eventually disappeared. Because the treatment aims at something in the host cells—as opposed to one of the viral proteins (which is how the protease inhibitors work)—it should be effective against all strains of hepatitis C.

Although the intervention was designed to last just four weeks (the infection eventually returned in all the treated patients), there is reason to believe that longer treatment with miravirsen will prove more effective. "The thought is that if you block the viral replication long enough, you can cure the disease," says Harry L. A. Janssen, a senior scientist at the Toronto General Research Institute and a co-author of the miravirsen study, which was published in the *New England Journal of Medicine*. Further tests are ongoing.

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At least 30%

of people with hepatitis C are not cured after their first round of standard treatment

ence how proteins were formed. The disruption of mRNA by short RNA molecules was coined RNAi, for RNA interference, and the latter molecules were given such names as siRNA, for small interfering RNA. Meanwhile a wide range of scientists realized that they might be able to deal with undruggable classes of proteins by moving the action further upstream, at the RNA level of the protein-manufacturing process.

To date, more than 200 experimental studies of either microRNAs or siRNAs have been registered through the U.S. government's database of clinical trials for the diagnosis or treatment of everything from autism to skin cancer. Among the most promising are treatments for Ebola virus, an extremely deadly pathogen that terrorism experts fear could be turned into a bioweapon, and hepatitis C, which has triggered long-lasting infections in about 150 million people around the world and is a major cause of liver cancer [*see box at left and box on next page*].

WHAT'S NEXT?

WHEREAS MEDICATIONS containing microRNA or siRNA are furthest along in the race to the clinic, another generation of aspiring starlets is now waiting in the wings. These potential medications would work even further upstream, on the DNA molecule itself. One of the approaches is based on CRISPR sequences found in the DNA of many single-celled organisms and was enthusiastically described in *Science* as the "CRISPR Craze." The other, which depends on the existence of molecules known as long noncoding RNAs, or lncRNAs, still faces some skepticism about its utility.

CRISPR stands for *c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats, which are oddly repetitive stretches of DNA found in bacteria and archaea (bacterialike organisms). These quirky sequences, in turn, interact with proteins known as CRISPR-associated, or Cas, proteins. Together CRISPR and various Cas proteins form a microbial defense system against viruses.

The proteins have one job—to cut DNA in two. They are guided to specific stretches of viral DNA by complementary strands of RNA. Where does the RNA come from? In a microscopic version of jujitsu, cells grab the RNA from the invading virus, turning it into a double agent that guides the Cas proteins to the exact spot where they need to cut.

Although CRISPR elements were first observed in bacteria in 1987, scientists started adapting the system to a wide range of animal, including human, tissue only in 2012. By creating their own guide strands of RNA, investigators could direct the Cas proteins to cut DNA molecules in the nucleus at very precise locations. In essence, they had turned the bacterial defense mechanism into a precision gene-editing tool.

Such exquisitely targeted technology could potentially revolutionize gene therapy—perhaps sooner rather than later.

Currently clinical investigators are only able to inject corrective DNA into patients with defective genes in a scattershot

DEFEATING NATURE'S TERRORISTS

An RNA-based treatment may stop the Ebola virus in its tracks

By Ferris Jabr

At first, people infected with the Ebola virus appear to have the flu—fever, chills, muscle aches. Then the bleeding begins. As the virus hijacks cells throughout the body to make copies of itself, it overwhelms and damages the liver, lungs, spleen and blood vessels. Within days organs begin to fail and many patients fall into a coma. Some outbreaks, primarily in Central and West Africa, have killed up to 90 percent of infected individuals.

That terrifying prognosis may be about to change. Using so-called small interfering RNA, or siRNA, Thomas W. Geisbert, now at the University of Texas Medical Branch at Galveston, and his many collaborators have devised a highly promising treatment that has saved the lives of six monkeys infected with the virus. As reported this past January, the treatment has also passed its first safety test in an uninfected human volunteer. One of Geisbert's collaborators, Ian Maclachlan of Burnaby, British Columbia–based Tekmira Pharmaceuticals, and his team have received a $140-million grant from the U.S. Department of Defense to develop the therapy further.

Working together, the scientists engineered an siRNA to prevent the Ebola virus from making a particular protein, without which it cannot replicate itself. "If you knock out that one, in theory you knock out everything," Geisbert says. The researchers also designed another siRNA to thwart manufacture of a second protein that the virus uses to weaken an infected individual's immune system. There is no danger of the siRNAs interfering with typical cellular duties because the targeted viral proteins do not exist in the cells of humans or other mammals.

Maclachlan and his colleagues encapsulated the lab-made siRNAs in little bubbles of fat that cells would readily transport across their membranes. Then they injected the preparation into several rhesus macaques, which had been infected with Ebola virus less than an hour earlier. In one study, two of three monkeys given a total of four doses of the treatment in the first week after exposure survived. In a second study designed to test the effectiveness of a higher dose, all four monkeys that received seven siRNA injections lived. Tests revealed that the treated monkeys had far fewer virus molecules in their blood than is typical for an infected animal. The macaques tolerated the siRNA injections well, and those that survived were still healthy 30 days later.

Outbreaks, primarily in Central and West Africa, have killed

up to 90% of infected individuals

The study was a "milestone," says Gary Kobinger of the University of Manitoba, who is working on a different Ebola treatment based on antibodies. He believes Geisbert and his team "are leading the effort toward clinical development."

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SCIENTIFIC AMERICAN ONLINE Watch an animation of short RNAs at work in the cell at ScientificAmerican.com/apr2014/rna

manner, hoping that at least some genetic material manages to start working in the right place. Fully developed CRISPR/Cas technology could change that by allowing researchers to choose precisely where a patient's DNA should be modified. "We're going to be seeing quite a few gene therapy trials using CRISPR in the next year," says George M. Church, a professor of genetics at Harvard Medical School, co-founder of Editas and scientific adviser to *Scientific American*. "It basically works right out of the box," he adds. "You can take it out of bacteria with minimal changes. Almost every guide RNA you'd want to make goes to a place that works. It's fast, and it's permanent."

Church expects that Editas will proceed to clinical trials quickly after first completing animal studies. Other recently launched CRISPR-centric companies include Caribou Biosciences and Egenesis.

Finally, the most controversial of the latest RNA discoveries concerns lncRNAs. First described in 2002, these unusually lengthy stretches of RNA originate in the nucleus and look, at first glance, as though they might be mRNAs except that they lack certain sequences of letters required to initiate the translation process.

What could the cell possibly want with all these extra RNA molecules? Some of them undoubtedly result from the transcription of earlier versions of genes that are now broken and no longer functional. (One of the more surprising discoveries of the genetic revolution is that almost all DNA found in the nucleus is transcribed, not just the parts that code for proteins.) Others are probably echoes of long-ago attacks by certain kinds of viruses that can incorporate their genetic material into a cell's DNA, allowing it to be passed on through subsequent generations.

Yet what if some of the lncRNAs represent a previously unsuspected way of regulating the expression of genes—one that does not require potentially dangerous mutations in the DNA or that does not depend on proteins to play the starring role? Think of the DNA as being folded like origami, says RNA researcher John Rinn of Harvard University. With two identical pieces of paper, you could make a plane or a crane, and lncRNA somehow pushes the DNA to make sure the steps occur in the right order. Just as a mistake in origami folding could render the paper crane wingless, too much noncoding RNA, for example, might trigger the growth of a tumor without a single mutation ever having had to occur in the genes of the cell.

Another possibility under investigation is that lncRNA molecules may attach themselves to different parts of a DNA molecule, changing the latter's three-dimensional shape and therefore exposing it to, or hiding it from, further activity.

An entire host of other noncoding RNAs have been proposed and are in various stages of being confirmed as important genetic regulators or dismissed as genetic ghosts. One of the difficulties of studying noncoding RNAs is precisely the fact that they do not give rise to proteins—which makes it harder to prove that they are doing something important. "I think it's just the early days yet," says John Mattick, a leader in research into noncoding RNA and director of the Garvan Institute of Medical Research in Australia. "There's a whole new world emerging here."

Meanwhile considering the broad range of RNA compounds that are being designed and tested brings up what may be the molecule's most appealing feature—its simplicity. Unlike proteins, whose three-dimensional structure must typically be characterized before drug developers can create effective medications, RNA basically consists of a two-dimensional sequence (leaving aside, for the moment, some of the shapes into which RNA molecules can fold). "It's reducing a three-dimensional problem, where the small molecule has to fit perfectly into the protein in a lock-and-key-type fit, to a two-dimensional, linear problem," Isis's MacLeod says. Thanks to the Human Genome Project, researchers already know the most important sequences in the genome. All they need to do is synthesize the complementary RNA strand, and they have created the bull's-eye for their efforts.

Figuring out how to put theory into practice is still a struggle, of course. But for now, at least, the magical glass slipper appears to fit. SA

An early trickle of financial interest has turned into a multibillion-dollar torrent. One start-up recently received **$43 million,** and a slightly older company scored **$700 million** to develop its pipeline of RNA medicines.

Christine Gorman *is a senior editor and* ***Dina Fine Maron*** *is an associate editor at* Scientific American.

MORE TO EXPLORE

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