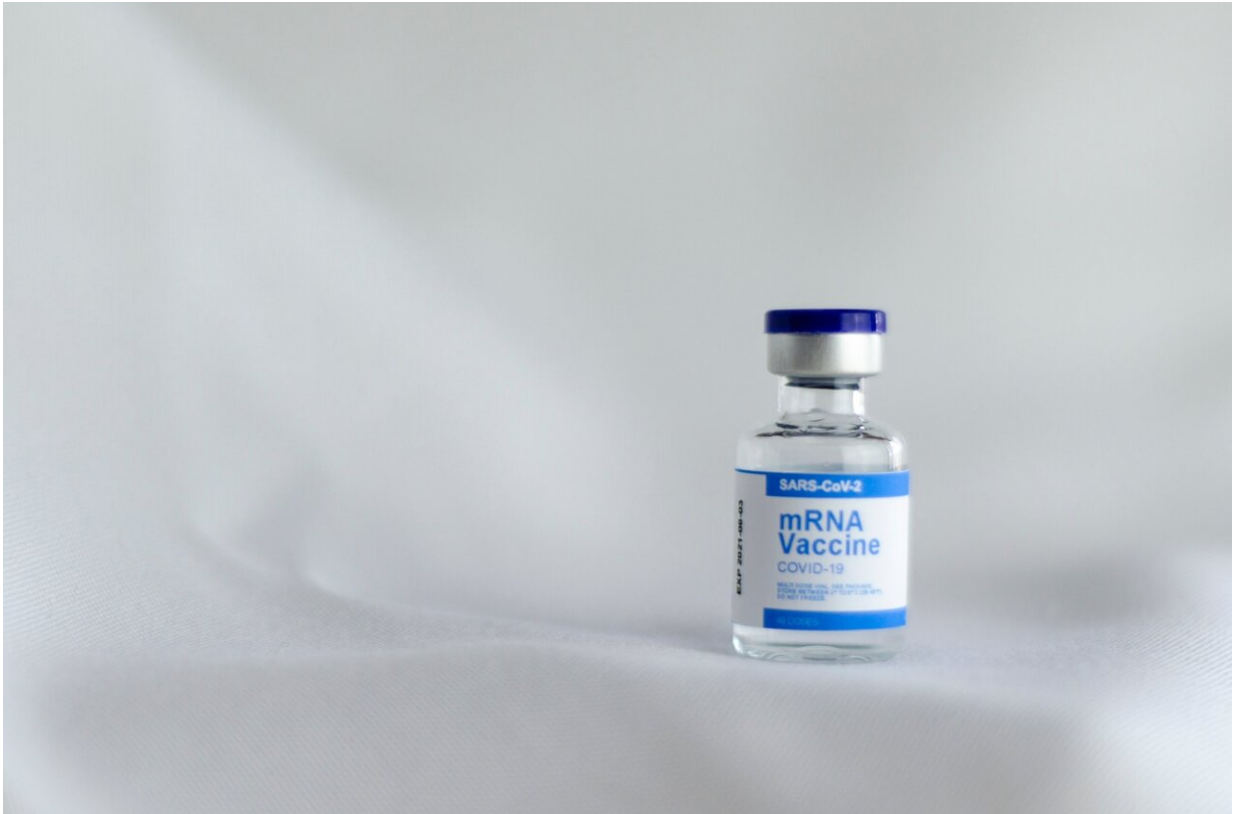


# mRNA has entered the lexicon

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Catastrophe occasionally apologizes for itself by coughing up a consolation prize. World War II gave us penicillin. So let's count our blessings.

The COVID-19 pandemic, from which we're still struggling to emerge,

has expanded our working vocabulary, gifting the public lexicon with new, if admittedly mostly gloomy, words and concepts. (Examples: [spike protein](#), intubation, N95, rapid antigen test.) We may not flood our speech with these terms, but we're at least passingly familiar with them now.

Emerging last but not least, like hope climbing gamely out of Pandora's box, is an exotic little acronym: mRNA. Once known only to biology majors, mRNA—more formally named messenger RNA—has entered society's word list, courtesy of a brand-new kind of vaccine.

Rarely has vaccine development exceeded the speed of paint peeling. But mRNA vaccines were hustled into commercial viability by Operation Warp Speed, a federal program set up in 2020 to accelerate the development of any vaccine that might stave off COVID-19's most severe symptoms.

Since receiving an emergency use authorization from the Food and Drug Administration in December 2020, many hundreds of millions of mRNA-based COVID-19 vaccine doses have been shot into people's arms in the United States alone. They've equaled or exceeded COVID-19 vaccines made through traditional means, with respect to both safety and efficacy. And they can be developed or modified with ease and rapidity. Today, scientists are developing mRNA vaccines for all kinds of other infectious diseases, as well as cancer.

There are solid reasons why mRNA may be a superior material for many vaccines, especially when pathogens keep swiftly evolving new strains. But realizing mRNA's full potential means addressing some non-negligible challenges, which Stanford University researchers are tackling. Among them: how to get more bang for the dose, how to send that dose to only where it's supposed to go and how to ensure it sticks around once it gets there.

The sudden appearance of a new kind of vaccine has generated concerns ranging from the spurious to the undeniable. For example, the shots can have side effects—a problem that may have a lot to do with how mRNA currently gets delivered and that, as we shall see, researchers are addressing.

The biggest source of mRNA-vaccine skepticism, according to vaccinologist Bali Pulendran, Ph.D., the Violetta L. Horton Professor and professor of microbiology and immunology, is rooted not in biology but in our own psychology—specifically, an amorphous, free-floating fear of the unknown.

"The human mind rejects any new idea like the body rejects a transplanted organ," he said.

## Making proteins

Unless you're a cell, making proteins is a tricky business. Just as different culinary creations require vastly different recipes—cooking vessels, timing, mixing methods and oven temperatures—every protein has its own peculiar manufacturing specs. That's a hurdle for fast production of traditional vaccines, which have specific proteins as key components.

Making proteins is easy for [cells](#), though. It's what they do. The [cell nucleus](#), home to our genome, doesn't let any of the 22,000-odd genes it encloses leave the premises. But those DNA-constituted genes can be copied in the form of smaller strands of RNA, a DNA-doppelganger substance that can exit the nucleus.

Every mRNA molecule's mission is to carry the genetic recipe for whatever protein it encodes (hence the "m" for "messenger") to the cytoplasm—all the cell's territory outside the nucleus. That's where

abundant numbers of a type of molecular machine—protein-printing presses called ribosomes—hang out. Ribosomes know how to read any mRNA recipe and cook up a batch of the indicated protein in a jiffy. These are well-oiled machines honed by eons of evolution.

The success of mRNA technology lies in outsourcing the heavy lifting of protein manufacturing to the ultimate protein factories: our own cells, which can host millions of ribosomes apiece.

Because biotechnologists can rapidly synthesize buckets of mRNA molecules specifying any desired protein, it's a reasonable strategy for creating a vaccine, pronto. (Proteins from pathogens, or chunks of those proteins, are what make up most vaccines. Exposure to them trains the immune system to launch an attack on that pathogen.)

Fine. So, making mRNA is a snap. But getting it to the right cells and, once inside, to the ribosomes—the key to gaining great protection with minimal side effects—requires ingenuity. Simply shoot naked mRNA into someone's veins and it will quickly get chewed up by enzymes in the blood or tissues. It's very delicate. And mRNA can't whiz effortlessly through cells' protective outer membranes.

You need a delivery vehicle.

## **mRNA's wild ride on the fat-blob express**

The COVID-19 mRNA vaccines have been ushered into our cells via workhorse delivery trucks called [lipid nanoparticles](#) (lipid being a scientific term for "fatty stuff"). Lipid nanoparticles, or LNPs, are glorified fat globules.

"An LNP is a crude attempt to do what a virus does for a living," said Stanford University professor of chemistry Bob Waymouth, Ph.D.

"Viruses are really good at getting inside cells so they can replicate themselves."

A lipid nanoparticle is a four-ingredient sphere roughly 100–200 nanometers in diameter (coincidentally, the size of the virus that causes COVID-19). Two ingredients stabilize the lipid nanoparticle's chemical composition. A third prevents lipid nanoparticles from clumping up, as fat blobs are inclined to do. The fourth, linchpin, ingredient is a bunch of linear, fatty molecules carrying a generally positive electrical charge along their lengths.

An mRNA strand is negatively charged along its length. And, as we all know, opposites attract, particularly in electronics. So, the two stick together, anchoring the mRNA to the lipid nanoparticle. A single lipid nanoparticle can encase multiple mRNA molecules.

Lipid nanoparticles are formulated to deliver their mRNA cargo safely into cells and release it. This frees the mRNA molecules to skedaddle into the cytoplasm and clamber onto its resident ribosomes. But in practice, only around 10% of mRNA smuggled into a cell by lipid nanoparticles ever winds up producing proteins.

"LNPs have a problem with letting go," Waymouth said.

Another problem: Most lipid nanoparticles never get to the desired cells in the first place. Once injected, lipid nanoparticles tend to gravitate toward certain organs and cell types. Left to their own devices after intravenous injection, the vast majority head for the liver—super, if you're trying to medicate liver cells. (Companies all over the world are working on mRNA-based medicines designed for this purpose, Waymouth said.)

Otherwise, not so great.

COVID-19 shots are, of course, injected into muscle tissue, not veins. Even so, some of the lipid nanoparticle-borne mRNA lands in the liver, animal trials suggest. More gets into muscle cells. Small amounts wind up in still other places, which Waymouth said could cause some of the side effects of the vaccines.

Fortunately, a fair amount of it reaches front-line sentinel "show and tell" cells of the immune system that hang out in muscle tissue or nearby lymph nodes. These immune cells are ideal vaccine targets. They gobble up lipid nanoparticles, follow the ingested mRNA's instructions and make proteins, which they chop up into little pieces and display on their surfaces for other immune cells to recognize as foreign material—a key step in kicking a coordinated immune response into gear.

Lipid nanoparticles aren't overly toxic in themselves, but they do cause some inflammation and are suspected of being responsible for some of the more common COVID-19 vaccine side effects, such as sore arms, fever and redness.

Some side effects are more worrisome—for instance, myocarditis, a rare inflammation-driven heart problem experienced primarily by young men and adolescent boys. Whether lipid nanoparticles' inflammatory potential, plus their tendency to wander off, might contribute to some of these less frequently observed, but troubling, COVID-19 vaccine-associated symptoms is an open question. (Having COVID-19 itself imparts a greater myocarditis risk.)

It's a reasonable bet that if biomedical scientists could direct mRNA to targeted cells or organs and nowhere else, this might lower the risk of side effects. What's inarguable is that less of the vaccine would need to be injected. With less going to waste, there'd be more to go around. That's worth considering when you're trying to vaccinate the world's whole population, or close to it, all at once.

There may be a way to do just that. Newer, more efficient, more targeted ways of packaging mRNA could provide a powerful boost for its expanded use in treating conditions beyond COVID-19.

## Shopping for CARTs

Waymouth and chemistry professor Paul Wender, Ph.D., have been working together for more than a decade on simplified, more efficient mRNA delivery vehicles. These vehicles are called charge-altering releasable transporters, or CARTs.

CARTs (described in a January 2017 paper in the Proceedings of National Academy of Sciences) are little spheres about the same size as lipid nanoparticles. But CARTs are not mere fancy fat globules. They consist mostly of a single stringy substance that's part fat and part protein-like. As with lipid nanoparticles, molecules of this substance are positively charged, so they can hang onto mRNA, which has a negative charge.

Fine-tuning the makeup of CARTs—say, by swapping out one type of building block for another—can radically change where they go in the body. Constituted one way and injected intravenously, they'll almost all head for the spleen, an organ practically bursting with immune cells awaiting orders to attack any pathogen that crosses their path. That's a plus for any scientist hunting for a new way to deliver potent vaccines.

Constituted just a little differently, CARTs will mainly lunge for the lungs.

"Where they go depends on what they're made of," said Wender, who's working with Waymouth on additional variations that could expand the list of designated destinations in the body. That list could come in handy for, say, developing a gene therapy meant to induce production of a



particular protein in a single organ or cell type.

## **mRNA 2.0: Special-delivery service**

When CARTs are administered intramuscularly (in mice, anyway) they mostly stick around the injection site until local immune cells spot and ingest them. Or, even better, the CARTs head for a nearby lymph node where teams of immune cells suck them up and initiate a response.

Once inside any cell, immune or otherwise, a CART doesn't wait around long before its wheels come off. It literally falls apart. Soon after gaining entry to a cell, its hybrid fat/protein-like carrier molecules lose their positive charge, release their grip on the cargo they were holding—mRNA strands—and break into tiny pieces.

"We designed them that way," Waymouth said.

The overarching result, said Wender, is that "the mRNA molecule gets released on time, virtually all the time." In other words, the CARTs let go of their mRNA cargo in the right place and in good condition, and that's the recipe for the successful delivery of any medicine or vaccine.

Broken down, CARTs' pieces are nontoxic, as are intact CARTs. In fact, CARTs are non-immunogenic, meaning you actually have to add immune-stimulating enhancements to rev up the immune response.

Waymouth suggests the inert nature of CARTs makes them potentially tunable. "This might allow us to dial in the particular immune response we want to induce," he said. "Keeping it immunologically inert," he added, "would prevent multiple injections over time from triggering an unwanted inflammatory response."

In 2021, Wender, Waymouth, professor of oncology Ronald Levy, MD,



and coworkers conducted [a study](#) to test this idea, the fruits of which were published in *ACS Central Science*. Onto mRNA-filled CARTs, they loaded an additional stretch of genetic code. This stretch, common among viruses, alerts intracellular receptors to the presence of microbes within a cell. Its inclusion substantially boosted the immune response to the mRNA-encoded protein in mice—a promising sign for future vaccine development.

Levy, who is widely known for his innovations in cancer immunotherapy, began collaborating with Waymouth and Wender a half a decade ago. In [a mouse study](#) published in 2019 in *Cancer Research*, they injected a CART-drawn combo of mRNA snippets directly into a tumor. The three snippets were recipes for three well-known immunity-stimulating substances.

The researchers witnessed a strong immune response not only to the tumor they'd injected with the immune-stimulating mRNA—a not entirely unexpected result—but also to a separate, similar tumor located elsewhere in the body, suggesting that this method might someday have application in ridding a cancer patient of metastases.

## **Bigger bang theory**

A standard, homemade mRNA molecule doesn't last long inside a cell. It's not built to last. Otherwise, a cell would keep cranking out proteins after they were no longer needed. Enzymes inside cells prevent that by biting off an mRNA molecule's ends piece by piece, like whittling down a stick of beef jerky.

But an RNA molecule whose ends have been joined to form a ring is impervious to those chomp-and-chew enzymes.

Seeking a bigger bang for the dose, Waymouth and Wender have been

collaborating with professor of dermatology and of genetics Howard Chang, MD, Ph.D., a world expert in exploring and exploiting circular RNAs, or circRNAs. These are, as the name implies, single RNA molecules joined head to tail, enhanced with built-in entry sites that ribosomes—cells' protein-printing presses—can grab onto. Once they've snagged the mRNA, they can spew out proteins abundantly and for longer. (Picture a single mRNA molecule spinning around and around like a vinyl disk on a turntable.)

A [paper](#) by Chang, Wender and other researchers, published online in *Nature Biotechnology* in April 2022, showed they could cobble together circRNA that stays stronger for longer. It resists degradation on its way to cells, yields copious copies of the protein it encodes, and lasts longer inside cells before breaking down.

"In our own work, linear mRNA typically survives inside of a cell for about 24 hours," Wender said. "By 48 hours, it's gone." But, the *Nature Biotechnology* paper showed, in mice, circRNA delivered on CARTs lasted inside the cells it penetrated for more than seven days. So a single molecule of it could churn out a lot more of the desired protein than if it were more ephemeral.

CARTs have yet to be tested in humans in rigorous clinical trials. That looks to be a few years off—although, as we've seen with COVID-19, perceived need can vastly accelerate treatment.

"CircRNAs can also be packaged and delivered in lipid nanoparticles," noted Chang, the Virginia and D.K. Ludwig Professor of Cancer Research. Some companies developing circRNA medicines are using lipid nanoparticles as delivery vehicles, he said.

Whether delivered by lipid nanoparticles, by CARTs or by some yet-to-emerge breakthrough technology, mRNA is going to play an increasingly

prevalent role in [vaccine development](#).

## Everything everywhere all at once

COVID-19 was the launchpad for mRNA vaccine technology. As the world emerges from the worst of the pandemic, can the same platform dispatch mRNA vaccines aimed at pretty much any microbe of choice? Pulendran, the vaccinologist, answers with a resounding yes.

"Any vaccine you can think of, the mRNA-frontrunner companies are working on it," said Pulendran, who has consulted with BioNTech, Moderna and Pfizer, three companies closely associated with the technology. "Their world view is that mRNA technology will replace all preceding ones. The future is extremely bright for mRNA vaccines."

Pulendran noted a couple of criticisms that have been leveled at the mRNA-based COVID-19 vaccines. "They've been very good at preventing severe disease, hospitalization and death," he said. "So far, they haven't been so good at preventing infection for long periods of time—particularly in the face of ever-newer viral variants—and they haven't been great at preventing transmission."

But transmission is a problem common to all respiratory infections, he said. It's tough to completely prevent infection of the nose and throat with any vaccine, since these outward-facing cavities' cells are constantly exposed to the air—and, consequently, the microbes—we inhale.

"To me, the most critical goal of a vaccine is to prevent severe or even moderate disease," Pulendran said. "A mild COVID 'cold' may even benefit us by keeping our [immune system](#) on its toes." On the other hand, he said, mRNA vaccines employing delivery systems that target the mucus-secreting linings of our airways and gut may prove more effective at durably preventing infection.

Was the technology that rescued those most susceptible to depredations of SARS-CoV-2—the virus that causes COVID-19—a one-hit wonder, or was it a cornucopia conferring protection from microbial menaces of every stripe? Time will tell.

And it won't take long. Clinical testing is underway for mRNA-based vaccines for influenza, HIV, cytomegalovirus, dengue, rabies and several other viruses, as well as for malaria, tuberculosis and other non-viral microbes. Moderna is submitting its mRNA-based vaccine directed at respiratory syncytial virus to the FDA for approval. Moderna and Merck are collaborating on a personalized skin-cancer vaccine employing mRNA, now in clinical trials.

These are all familiar, if uninvited, maladies. What humanity should most fear—and what the plug-and-play mRNA [vaccine](#) technology promises to provide the most valiant and rapid defense against—are those next unfamiliar monsters climbing over the hill toward us, which science-fiction movies always seem to depict as giants but are actually microscopic.

Provided by Stanford University

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