Foreword

July 20, 1969. Around the world, 650 million people were glued to their television sets, watching Neil Armstrong emerge from the lunar lander and take the first-ever steps on the moon. As they watched, they were in awe of the long years of behind-the-scenes work required to perfect rockets and space capsules and to compute trajectories, and they celebrated the moon landing as a heroic achievement of science and engineering.

I was one of the millions watching. Little did I imagine at the time how my own research career would take me in the opposite direction. I would, in a sense, be looking through the other end of the telescope. Instead of exploring the vast enormity of outer space, I would revel in observing and manipulating miniscule molecules that work in living cells. I would witness firsthand discoveries about RNA—ribonucleic acid—that would cause scientists to rethink the deep question of how life originated on planet Earth and would produce breathtaking opportunities for human health and treating disease. My research group and I would make some of these discoveries ourselves, and I would be standing nearby as friends and colleagues made many of the others. And seeing the astounding feats performed by RNA would be more thrilling to us than any moonwalk.

But these remarkable advances in RNA research would go largely unknown by the general public. The same people who applauded the moon landing had no idea that an equivalently thrilling adventure was taking place in RNA laboratories around the world. Minute volumes of RNA solutions were being pipetted into test tubes, drops of enzymes added, the products tested in human cells and in living organisms—yeast and worms and fruit flies and mice. All of this work being done not for medical application, but for the thrill of understanding how nature works. Yet, inevitably, this curiosity-driven research would build the framework for what is arguably the greatest scientific achievement since the moon landing.

And then, very suddenly, after decades of obscurity, the three letters R, N, A were being tossed about by everyone. Spurred on by early signs that there might be a worldwide COVID-19 pandemic, many biotechnology and pharmaceutical companies raced to develop a vaccine. Although most of the companies tried to repurpose traditional methods for vaccine development, BioNTech in Mainz, Germany, and Moderna in Cambridge, Massachusetts, were racing to make the first messenger RNA (mRNA) vaccines. This was very bold—targeting a just-discovered virus, about which we knew very little, with a never-before-tested vaccine technology.

After 10 months of intense effort, the two companies unblinded their placebo-controlled trials to learn that their vaccines were 95% effective. In other words, for every 100 subjects who got COVID-19 during the trial, 95 were in the placebo group (receiving shots of saltwater) and only five were in the vaccinated group. This was cause for wild celebration. The very first mRNA vaccines were not only successful but also extremely potent. These mRNA vaccines prevented perhaps 20 million COVID-19 deaths worldwide in their first year, as recently calculated by researchers from Imperial College London. Although such estimates can only be approximations, the answer is clear: mRNA saved millions of lives.

How did it start? It was January 10, 2020, when the sequence of A, G, C, and U bases in SARS-CoV-2 RNA was revealed on the Internet by a group of community-minded Chinese scientists. That information provided the code for the virus' spike protein. This was an essential starting point for mRNA vaccine development, because to design an mRNA that codes for any particular protein, you need to know the base sequence. Starting in January, it took the two companies less than a year to design, develop, manufacture, and test their mRNA vaccines—and get them approved for emergency use. This not only set the speed record for vaccine development but also eclipsed all previous timelines. For comparison, traditional vaccines take 5 to 8 *years* to develop and test.

Traditional vaccines prime the immune system by presenting it with an inactivated or replication-defective version of a virus. (Indeed, two Chinese companies developed just such vaccines against SARS-CoV-2.) It is difficult, expensive, and dangerous to produce large quantities of a deadly coronavirus to make a vaccine. So, the mRNA vaccines take a shortcut. If it's just the spike protein that human immune cells need to recognize,

why do you need the rest of the virus? Simply inject an mRNA encoding the spike protein and let our immune cells use it to make the protein. This RNA represents only a small part of the coronavirus genome, so it cannot cause infection by itself—an enormous safety advantage.

Sounds easy, right? But the devil was in the details. RNA is notoriously unstable—enzymes that chew up RNA, called ribonucleases, are everywhere—and large, negatively charged molecules are repelled by the plasma membrane that surrounds cells, so they can't get in. Thus, encapsulating the mRNA in lipid nanoparticles was key. Furthermore, the vaccine mRNAs needed to be stabilized by the end-capping groups that decorate the ends of normal cellular mRNAs. On top of that, humans have an ancient surveillance system called the innate immune system that is on the lookout for incoming RNA, because incoming RNA usually spells "virus." It sets off protective reactions that sometimes overheat, causing inflammation and pain. But clever biochemists found that they could decorate the U letter of the RNA alphabet with chemical groups that kept it from being detected by the innate immune system, and yet it still retained its ability to code for the spike protein.

Remarkably, these decades of prior research—which set up the mRNA vaccine projects for success—were not in most cases motivated by curing a disease. The research scientists were not aiming to launch a biotech company or to establish a novel platform for drug development. Instead, they were just curious about how nature worked. One great example is the "in vitro transcription" process that both BioNTech and Moderna used to synthesize their mRNA. ("Transcription" is the act of copying DNA into RNA, and "in vitro" is Latin for "in glass," a descriptor that has survived the transition from glass test tubes to plastic ones in laboratories.) First, scientists interested in the workings of the T7 bacterial virus discovered the enzyme that copies its DNA into RNA. When they announced that it was a powerful RNA-making machine, other scientists harnessed it to study various roles of RNA in biology. As a 40-year-old example from my own work, when we were studying a catalytic RNA that performed cutting-and-pasting reactions on itself, we synthesized the RNA by T7 in vitro transcription.

Thus, research scientists studying the basics of RNA paved the road for the vaccine makers. They found all the puzzle pieces and laid them out on the table. It was then up to the vaccine companies to find which knobs

went in which sockets and put all the pieces together—still a daunting task, but one that would have been impossible without the prior research. Fabrice Delaye's book celebrates the RNA science that made the mRNA vaccines possible. It's so fitting that the scourge of the SARS-CoV-2 RNA virus was a vaccine made of RNA.

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Delaye's account of messenger RNA medicines is remarkable in several ways. As a nonscientist, Delaye has worked diligently to understand the concepts of molecules and cells, of DNA, RNA, and protein, of lipids and nanoparticles. Because he remembers how he struggled to understand these concepts, he tailors his descriptions to help his nonscientist readers overcome the hurdles.

Furthermore, Delaye is unabashedly enthusiastic about mRNA. Even as he relates all the years that mRNA went unappreciated and was disparaged as a potential pharmaceutical, Delaye is in the stands of the stadium rooting for team RNA. As an RNA scientist, I find this enthusiasm for RNA to be obvious, and when I attend an RNA conference, the feeling is unanimous. Yet it is exciting to see a nonscientist so enthusiastic about the bashful daughter of DNA.

Finally, Delaye tells stories about the scientists making the discoveries as much as the research itself. An unruly lot these mRNA scientists are! If the reader has been envisioning scientists as calm, studious eggheads moving about calmly in white laboratory coats, that view will be debunked in every chapter. Delaye relates how scientists deal with the disappointment and setbacks that inevitably accompany research at the frontiers of knowledge. He describes how competition between laboratories simultaneously speeds progress and enrages some of the competitors. And how, when the COVID-19 pandemic arrives and the health of the world is at stake, the intense competition is tempered by some timely cooperation.

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Where will stabilized mRNA therapeutics go from here? Are we sitting at an inflection point in pharmaceutical science, where mRNA is on the

brink of providing new therapies for numerous diseases? Will there be an mRNA revolution as big as the monoclonal antibody revolution, which transformed medical therapy starting in the 1990s? Or, on the other hand, will mRNA prove to be useful mainly in the vaccine space and not invade the domains currently ruled by small-molecule drugs and antibodies?

A conservative prediction is that the COVID-19 mRNA vaccines have paved the way for additional mRNA vaccines. This by itself could satisfy substantial unmet medical needs. As one example, influenza (flu) vaccines have limited efficacy because they can't match the diversity of the flu virus. Flu vaccines are mostly made by hand-injecting a vaccine strain of the virus into chicken eggs. The long timeline for vaccine production prevents the vaccine from being tailored very accurately to the strains of flu virus that emerge in a given year. The speed and flexibility of mRNA vaccine development could produce vaccines more tightly matching each year's virus, potentially leading to high efficacy. Another exciting possibility is a single mRNA vaccine that works against all strains.

The bigger question is how much of an impact stabilized mRNA therapies will have outside of the vaccine space. Although many traditional pharmaceuticals act by inhibiting a disease-promoting process, mRNA is useful in the other direction—it adds back a healthy protein that is missing or mutated in a patient. Cancer and autoimmune disease are areas in which expressing a protein could be therapeutic, and mRNA scientists are already pursuing these. In fact, they were pursuing them before they got distracted (much to our benefit) by making COVID-19 vaccines.

Beyond that, in numerous genetic diseases, a protein is missing or mutated, and expressing the natural active protein may ameliorate the condition. Examples include muscular dystrophy, cystic fibrosis, and spinal muscular atrophy. Furthermore, there is a virtual universe of so-called rare diseases, each of which afflicts sometimes 1000 or 10,000 people worldwide; this represents too small a market for a pharma company to recoup the enormous cost of drug development and clinical trials. Now with mRNA therapies, there is a glimmer of hope that we might be able to have a single mRNA therapeutic platform into which we'd insert any of an enormous number of coding sequences, personalized to the patient.

Finally, therapeutic "monoclonal" antibodies represent a very large share of new pharmaceutical agents. They are currently delivered as

proteins, which requires expensive, time-consuming, and sometimes painful intravenous infusion. Because every protein has a corresponding mRNA, could these antibodies be delivered as stabilized mRNAs, by subcutaneous injection—just like a vaccination? Perhaps it won't be possible to produce a therapeutic dose of an antibody via the mRNA route, but the idea is attractive enough that scientists are currently exploring it.

Beyond messenger RNA, there is an entire universe of noncoding RNAs. "Noncoding" means that these are not mRNAs; they do not specify proteins. Instead, they are movers and shakers inside cells—either as critical components of biocatalysts or as regulators of gene expression. Might these be manipulated to therapeutic advantage? Might there be a "Medical Revolution of Noncoding RNAs" to complement the medical revolution of messenger RNA? My own research group is one of hundreds of academic laboratories around the world working on these noncoding RNAs, and in my forthcoming book on RNA (W. W. Norton, 2024), I'll aim to tell their story beside that of mRNA—both the biology and the medical possibilities. Alongside Delaye and the rest of "team RNA," I relish every opportunity to spotlight this miraculous molecule for the public. After all, the medical moonshot of the COVID-19 mRNA vaccines sets the launchpad for more thrilling RNA journeys to come.

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