and months after a single administration," according to CEO Diego Miralles. "We believe this could have a huge impact in how we think about protein therapeutics, opening a whole world of possibilities in how we treat diseases and relieve human suffering," he says.

The idea of using engineered circular RNAs as potential therapeutic agents dates back more than 25 years, but the strategy never gained much traction back then. Virologist Peter Sarnow, for example, filed for some early intellectual property around the platform after showing in 1995 that circular RNA could prompt protein production in human cells, but his institution at the time, the University of Colorado in Denver, discontinued the patent because there was no commercial interest. "Bummer!" says Sarnow, now at the Stanford University School of Medicine.

More recently, as the limitations of linear mRNA have become apparent, entrepreneurs are taking a second look at RNA circles. "It really has caught people's fancy now in ways it didn't before," says Manny Ares, an RNA biologist at the University of California, Santa Cruz, who in the 1990s developed some of the first methods for synthesizing circular RNAs.

In addition to the prolonged expression dynamics of circular RNA, Orna CEO Tom Barnes points to several other benefits of the platform compared with linear mRNA. For starters, the manufacturing is more cost effective: "Because the RNAs autocatalytically circularize at high efficiency," he says, there is no need for further steps with expensive reagents. "There is no capping, there is no tailing" —referring to chemical modifications at the 5′ and 3′ ends of mRNA molecules.

Circular RNAs can also evade the innate immune responses with or without the addition of modified nucleotides, thus adding further cost savings. Plus, Barnes notes, the technology can accommodate long sequences—up to 5 or possibly even 10 kilobases, as Anderson and Alexander Wesselhoeft, Orna's cofounder and director of molecular biology, have shown—which could open up new therapeutic possibilities.

"To me, it's clearly better," says Robert Kruse, a physician-scientist at Harvard Medical School in Boston, of circular RNA. But even though Kruse holds patents around the technology, he remains circumspect about whether the platform offers much more than incremental advances over linear mRNA. "The half-life is a great advantage. But beyond frequency of dosing," he says, "I don't know whether it will truly unlock a new therapeutic application."

In the meantime, some companies are findings new therapeutic applications for mRNA simply by reimagining what is possible with existing technologies. At Intellia Therapeutics, for example, the company's in vivo gene-editing therapies are all based on mRNAs that encode the Cas9 endonuclease enzyme. (Others have traditionally used viral vectors or ribonucleoprotein complexes to deliver the CRISPR machinery instead.)

The company reported in June results from six patients with the rare and fatal condition transthyretin amyloidosis who received infusions of the genome-editing RNA construct. The data showed that the mRNAs were successfully transformed into active Cas9 proteins that cut DNA at a specific target sequence, the location determined by an engineered guide RNA that was co-formulated alongside the mRNA inside liver-targeted lipid nanoparticles. The therapy introduced frameshift mutations at the disease-associated gene in liver cells, leading to declines in circulating levels of the aberrant protein.

Notably, in this context, the fleeting nature of mRNA is desirable, not a drawback. "I just want to have a pulse of Cas9 protein being made," says Laura Sepp-Lorenzino, CSO of Intellia. Otherwise the enzyme could spark immune reactions or off-target effects. "For me," she says, "short duration of expression is exactly what I'm looking for."

The same goes for Geoffrey von Maltzahn, CEO of Tessera Therapeutics and a general partner at Flagship Pioneering. Tessera is using a protein borrowed from the retrotransposition machinery of mobile genetic elements to make targeted insertions of therapeutic sequences into the genome. In the company's preclinical-stage products, that protein too is encoded in mRNA. "We're in the year of nucleic acids," von Maltzahn says, "and there's a ton of synergy" between different platform technologies. "Brought into the realm of gene writing and the broader area of genomic medicine, it's our view that the future is definitively going to be powered by RNA therapeutics."

Sanofi and others are betting on that future as well.

As Ehlers points out, targeted delivery of mRNA to most organs and tissues remains a challenge, which "will limit applications and indications." But, he says, "we see lots of reasons for optimism." RNA-based drugs are "going to be a big therapeutic class—no question." ❐

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CRISPR-based portable COVID tests

Two new CRISPR-based tests developed by academic groups have each taken a step toward providing portable, low-cost, sensitive SARS-CoV-2 diagnostics. A team at MIT and the Wyss Institute led by James J. Collins, publishing in *Science Advances*, describes an at-home saliva test that detects and reports the presence of different viral variants within an hour. The other COVID-19 diagnostic, published in *Nature Chemical Biology* by Jennifer Doudna's group at the University of California, Berkeley, is a point-of-care diagnostic that enables results using swab testing within 20 min.

The MIT team developed the miSHERLOCK (short for minimally instrumented SHERLOCK) test. It is based on the CRISPR platform developed by Sherlock Biosciences (co-founded by Collins and Feng Zhang from the Broad Institute). The new assay uses Cas12a guide RNA to distinguish different variants with a limit of detection comparable to that of the gold-standard RT-PCR test: 1 molecule per microliter in unprocessed saliva. The team aimed to show it was possible to build a low-cost CRISPR-based test involving minimal instrumentation to detect SARS-CoV2 in raw samples, with results delivered to a smartphone app.

The competing paper from the Doudna lab combines two unrelated CRISPR nucleases—RNA-guided Cas13 and Csm9—in a tandem assay to provide a simpler alternative to PCR-based methods in a portable microfluidic chip. The one-step assay, named Fast Integrated Nuclease Detection in Tandem, differs from other approved CRISPR-based COVID assays in that it does not require an amplification step. The tandem nuclease technology boosts detection and amplifies the signal such that it can detect 30 copies per microliter of target RNA, well below the threshold of 100 copies per microliter necessary for diagnostic surveillance.

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