Researchers track the lifespan and myriad functions of mRNA

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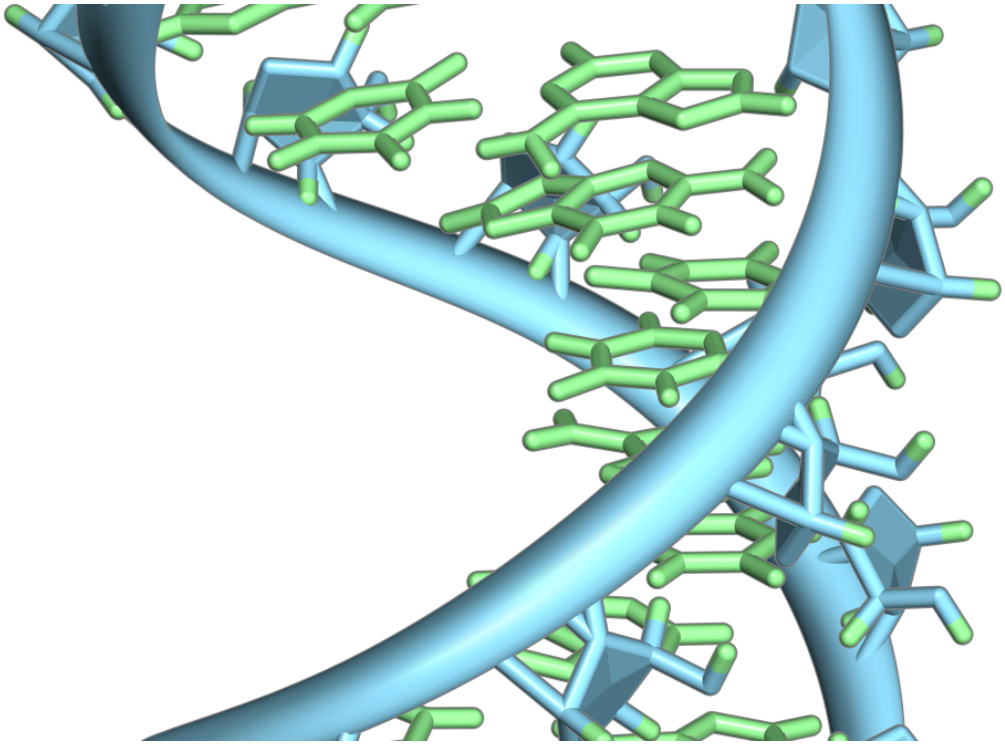

A hairpin loop from a pre-mRNA. Highlighted are the nucleobases (green) and the ribose-phosphate backbone (blue). Note that this is a single strand of RNA that folds back upon itself. Credit: Vossman/ Wikipedia

It took a global pandemic, but the critical role of messenger RNA in all of life's functions has taken center stage in the past year with the successful rollout of mRNA vaccines to combat the SARS-Cov-2 virus.

In two new papers published the week of Jan. 17, the lab of Yale's Wendy Gilbert sheds light on how mRNAs are born and how they regulate production of proteins inside of our cells once they reach maturity. The findings have implications not only for achieving effective doses for new vaccines, but for helping determine the biological roots of many cancers and diseases.

"It's been exciting to be able to study the beginning and end of this process," said Gilbert, associate professor of molecular biophysics and biochemistry.

In classic textbook biology, cells precisely copy or transcribe genes encoded in DNA into mRNAs, which then ferry those instructions to the ribosome, the machinery within the cell that makes the proteins that carry out almost all life functions. This key role played by mRNA has made the molecule a major research target for decades, including research that led to the rapid development of mRNA vaccines in the fight against COVID-19. The vaccines developed by Pfizer and Moderna contain mRNA-based instructions for cells to produce proteins that recognize spike proteins on the surface of the SARS-Cov-2 virus, making them targets for destruction by the immune system.

While RNA is formed from just four bases, or nucleotides, its structure and function can be altered by complex biochemical interactions with other compounds. One such compound that modifies mRNA is pseudouridine, an isomer whose presence is a key to the effectiveness of mRNA vaccines. Even before the pandemic, Gilbert's lab discovered the presence of pseudouridine in normal cellular mRNA. At the time, she became curious about how exactly these mRNA modifications are created and how they affect the mRNA's function.

In one of the new studies, a team led by Nicole Martinez, a postdoctoral fellow in Gilbert's lab, found that pseudouridine plays a key role in the

genesis of mRNAs. The team found pseudouridine present at the earliest stages of the formation of mRNAs. And the researchers discovered evidence that it guides the splicing of genetic material that creates mRNAs, which in turn regulate gene activity, they report Jan. 19 in the journal *Molecular Cell*.

These findings shed new light on origins of diseases linked to variants of pseudouridine such as mitochondrial myopathy, digestive disorders, intellectual disability, and resistance to viral infection. For instance, several cancers have been linked to elevated levels of pseudouridine, suggesting that faulty splicing of mRNAs may trigger tumor formation and cancer metastasis.

In a second paper, published Jan. 17 in the journal *Cell Systems*, Yale researchers investigated just how it is that ribosomes know how many proteins to produce from the genetic instructions they receive from mRNAs. For the study, a team headed by Rachel Niederer, an associate research scientist in Gilbert's lab, developed new technology called direct analysis of ribosome targeting (DART) to find regulatory elements that can spur and silence the production of proteins by ribosomes. Manipulating such elements in mRNAs—in this case, within yeast—allowed scientists to modify production of proteins by a thousand-fold, they report.

The ability to precisely manipulate protein production has immediate applications in adjusting doses in mRNA vaccines such as those used to combat COVID, the researchers say. Their work led to a $1.7 million grant from Pfizer to the Gilbert lab and that of Carson Thoreen, associate professor of cellular and molecular physiology at Yale.

Gilbert stressed, however, that the technology could also be applied to development of any protein-based therapies for a multitude of diseases.

More information: Rachel O. Niederer et al, Direct analysis of ribosome targeting illuminates thousand-fold regulation of translation initiation, *Cell Systems* (2022). DOI: 10.1016/j.cels.2021.12.002

Nicole M. Martinez et al, Pseudouridine synthases modify human pre-mRNA co-transcriptionally and affect pre-mRNA processing, *Molecular Cell* (2022). dx.doi.org/10.1016/j.molcel.2021.12.023

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