Simplifying RNA editing for treating genetic diseases

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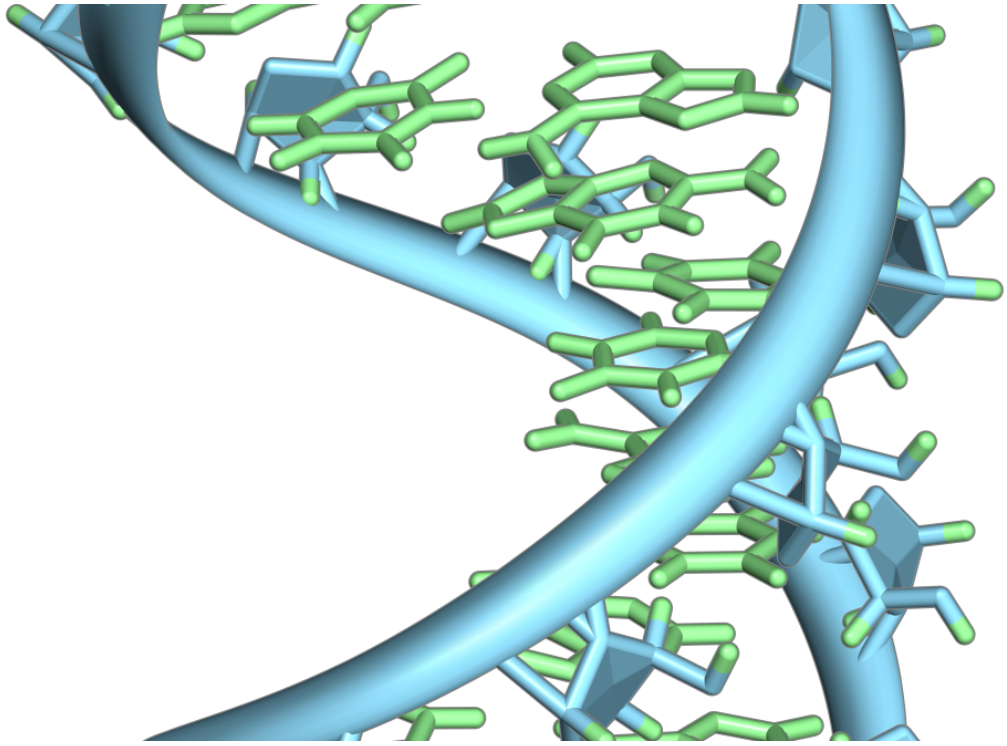

A hairpin loop from a pre-mRNA. Highlighted are the nucleobases (green) and the ribose-phosphate backbone (blue). Note that this is a single strand of RNA that folds back upon itself. Credit: Vossman/ Wikipedia

New research led by bioengineers at the University of California San Diego could make it much simpler to repair disease-causing mutations in RNA without compromising precision or efficiency.

The new RNA editing technology holds promise as a gene therapy for treating genetic diseases. In a proof of concept, UC San Diego researchers showed that the technology can treat a mouse model of Hurler syndrome, a rare genetic disease, by correcting its disease-causing mutation in RNA. The findings are published Feb. 10 in *Nature Biotechnology*.

What's special about the technology is that it makes efficient use of RNA editing enzymes that naturally occur in the body's cells. These enzymes are called adenosine deaminases acting on RNA (ADARs). They bind to RNA and convert some of the adenosine (A) bases to inosine (I), which is read by the cell's translation machinery as guanosine (G).

Researchers have been exploring RNA editing approaches with ADARs to correct the G-to-A mutation behind genetic disorders such as cystic fibrosis, Rett syndrome and Hurler syndrome. A big advantage of RNA editing—over DNA editing, for example—is that changes to RNA are only temporary, since RNA has a short lifespan. So even if off-target edits occur, they wouldn't be there to stay.

To make a targeted A-to-I (or essentially, an A-to-G) edit on RNA using ADARs, a short accessory strand of RNA—called a guide RNA—is needed to guide ADARs to the target and make the desired change there.

A big challenge with this approach is that traditional guide RNAs are not efficient at using native ADARs in the cell, so they require external ADARs to be brought into the cell to work, explained Prashant Mali, a bioengineering professor at the UC San Diego Jacobs School of Engineering. "But the problem with that," he added, "is that it makes delivery complicated. And it can result in more off targets."

To overcome these issues, Mali and colleagues engineered a new kind of

guide RNA—one that is extremely effective at recruiting the cell's own ADARs to make edits at a precise target RNA region.

"We can simply deliver just a small piece of RNA inside the cell and repair mutations *in vivo*. We don't have to provide any extra enzymes," said Mali.

The team designed the guide RNAs to target the single G-to-A mutation that causes Hurler syndrome. This mutation prevents the body from producing an enzyme that is necessary for breaking down complex sugars. Buildup of these sugars causes severe tissue damage, skeletal abnormalities, cognitive impairment, and other serious health problems. Systemic injection of the guide RNAs into diseased mice resulted in correction of 7 to 17% of the mutant RNAs after two weeks, as well as a 33% decrease in the buildup of complex sugars.

One aspect that makes the new guide RNAs effective is that they are longer than traditional guide RNAs. "This basically makes them stickier for ADARs already present in the cell to come and bind to them," said Mali. Other unique design features make them more stable and precise than traditional guide RNAs. They can last for days and stay on the target RNA region for longer periods of time, whereas RNA in general gets quickly destroyed by the cell. That's because these guide RNAs are built as circular rather than linear molecules; being circular makes them resistant to the cell's RNA-degrading enzymes. In terms of precision, these guide RNAs only allow changes at the target A and not at any other As nearby. They do this by folding into loop structures at predetermined spots along the target RNA region, which prevents off-target As from getting edited.

The research is still at an early stage, said Mali, "and it remains to be seen how this RNA editing technology will work in primates." Immediate next steps for the team will focus on improving delivery of

the guide RNAs into cells.

"I'm hopeful that this work opens the door even more for RNA editing as another gene therapy tool," said Mali.

A Seattle-based biotechnology startup co-founded by Mali, called Shape Therapeutics, is working to translate this and several other RNA editing technologies developed in Mali's lab into the clinic.

The paper is titled "Robust in vitro and in vivo RNA editing via recruitment of endogenous ADARs using circular guide RNAs." Co-authors include Dhruva Katrekar, James Yen, Yichen Xiang, Anushka Saha and Dario Meluzzi, UC San Diego; and Yiannis Savva, Shape Therapeutics.

More information: Prashant Mali, Efficient in vitro and in vivo RNA editing via recruitment of endogenous ADARs using circular guide RNAs, *Nature Biotechnology* (2022). DOI: 10.1038/s41587-021-01171-4 . www.nature.com/articles/s41587-021-01171-4

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