Discovery offers starting point for better gene-editing tools

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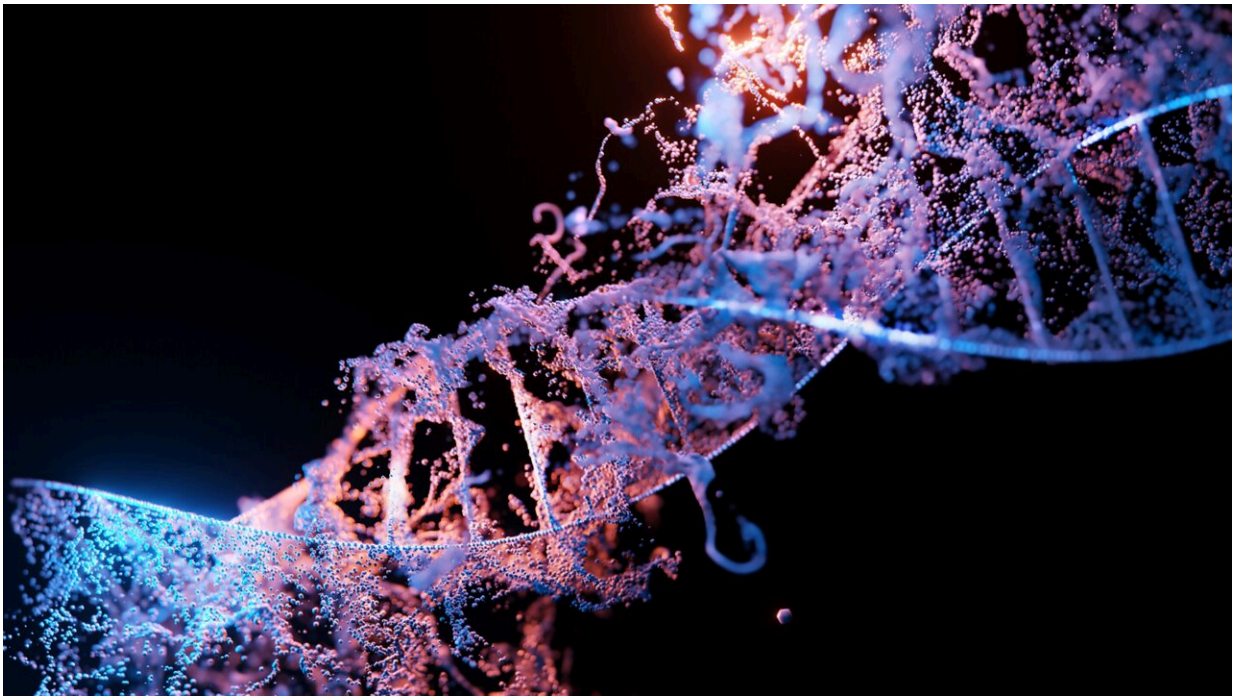

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CRISPR has ushered in the era of genomic medicine. A line of powerful tools has been developed from the popular CRISPR-Cas9 to cure genetic diseases. However, there is a last-mile problem—these tools need to be effectively delivered into every cell of the patient, and most Cas9s are too big to be fitted into popular genome therapy vectors, such as the adenovirus-associated virus (AAV).

In new research, Cornell scientists provide an explanation for how this problem is solved by nature: they define with atomic precision how a transposon-derived system edits DNA in RNA-guided fashion. Transposons are mobile genetic elements inside bacteria. A lineage of transposon encodes IscB, which is less than half the size of Cas9 but equally capable of DNA editing. Replacing Cas9 with IscB would definitively solve the size problem.

The researchers' paper, "Structural Basis for RNA-Guided DNA Cleavage by IscB-ωRNA and Mechanistic Comparison with Cas9," published May 26 in *Science*.

The researchers used cryo-electron microscopy (Cryo-EM) to visualize the IscB-ωRNA molecule from a transposon system in high resolution. They were able to capture snapshots of the system in different conformational states. They were even able to engineer slimmer IscB variants, by removing nonessential parts from IscB.

"Next-generation fancy applications require the gene editor to be fused with other enzymes and activities and most Cas9s are already too big for viral delivery. We are facing a traffic jam at the delivery end," said corresponding author Ailong Ke, professor of molecular biology and genetics in the College of Arts and Sciences. "If Cas9s can be packaged into viral vectors that have been used for decades in the gene therapy field, like AAV, then we can be confident they can be delivered and we can focus research exclusively on the efficacy of the editing tool itself."

CRISPR-Cas9 systems use an RNA as a guide to recognize a sequence of DNA. When a match is found, the Cas9 protein snips the target DNA at just the right place; it's then possible to do surgery at the DNA level to fix genetic diseases. The cryo-EM data gathered by the Cornell team show that the IscB-ωRNA system works in a similar way, with its smaller size achieved by replacing parts of the Cas9 protein with a

structured RNA (ωRNA) which is fused to the guide RNA. By replacing protein components of the larger Cas9 with RNA, the IscB protein is shrunken to the core chemical reaction centers which snip the target DNA.

"It's about understanding the molecules' structure and how they perform the chemical reactions," said first author Gabriel Schuler, a doctoral student in the graduate field of microbiology. "Studying these transposons gives us a new starting point to generate more powerful and accessible gene editing tools."

It is believed that transposons—mobile genetic elements—were the evolutionary precursors to CRISPR systems. They were discovered by Nobel Laureate Barbara McClintock.

"Transposons are specialized genetic hitchhikers, integrating into and splicing out of our genomes all the time," Ke said. "The systems inside bacteria in particular are being selected constantly—nature has basically tossed the dice billions of times and come up with really powerful DNA surgical tools, CRISPR included. And now, by defining these enzymes in high resolution, we can tap into their powers."

As small as IscB is compared to CRISPR Cas9, the researchers believe they will be able to shrink it even smaller. They've already removed 55 amino acids without affecting IscB's activity; they hope to make future versions of this genome editor even smaller and hence even more useful.

Better understanding the function of the companion guide RNA was another motivation behind the study, said co-first author Chunyi Hu, a postdoctoral researcher in the Department of Molecular Biology and Genetics. "There's still a lot of mystery—like why do transposons use an RNA-guided system? What other roles this RNA may be playing?"

One challenge that yet remains for the researchers is that while the IscB-ωRNA is extremely active in test tubes, it was not as efficient at altering DNA in human cells. The next step in their research will be to use the molecular structure to explore the possibilities they have identified for the cause of the low activity in human cells. "We have some ideas, a lot of them actually, that we are eager to test in the near future," Schuler said.

More information: Gabriel Schuler et al, Structural basis for RNA-guided DNA cleavage by IscB-ωRNA and mechanistic comparison with Cas9, *Science* (2022). DOI: 10.1126/science.abq7220

Provided by Cornell University

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