An ancient RNA-guided system could simplify delivery of gene editing therapies

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The Tas protein uses an RNA guide to recognize a specific target DNA sequence. Credit: The Tas protein uses an RNA guide to recognize a specific target DNA sequence. Credit: Max Wilkinson

A vast search of natural diversity has led scientists at MIT's McGovern

Institute and the Broad Institute of MIT and Harvard to uncover ancient systems with the potential to expand the genome-editing toolbox. These systems, which the researchers call TIGR (Tandem Interspaced Guide RNA) systems, use RNA to guide them to specific sites on DNA.

TIGR systems can be reprogrammed to target any DNA sequence of interest, and they have distinct functional modules that can act on the targeted DNA. In addition to its modularity, TIGR is very compact compared to other RNA-guided systems, like CRISPR, which is a major advantage for delivering it in a therapeutic context.

These findings appear in the journal *Science*.

"This is a very versatile RNA-guided system with a lot of diverse functionalities," says Feng Zhang, the James and Patricia Poitras Professor of Neuroscience at MIT who led the research. The TIGR-associated (Tas) proteins that Zhang's team found share a characteristic RNA-binding component that interacts with an RNA guide that directs it to a specific site in the genome. Some cut the DNA at that site, using an adjacent DNA-cutting segment of the protein. That modularity could facilitate tool development, allowing researchers to swap useful new features into natural Tas proteins.

"Nature is pretty incredible," remarks Zhang, who is also an investigator at the McGovern Institute and the Howard Hughes Medical Institute, a core member of the Broad Institute, a professor of brain and cognitive sciences and biological engineering at MIT, and co-director of the K. Lisa Yang and Hock E. Tan Center for Molecular Therapeutics at MIT.

"It's got a tremendous amount of diversity, and we have been exploring that natural diversity to find new biological mechanisms and harnessing them for different applications to manipulate biological processes," he says.

Previously, Zhang's team had adapted bacterial CRISPR systems into gene-editing tools that have transformed modern biology. His team has also found a variety of programmable proteins, both from CRISPR systems and beyond.

In their new work, to find novel programmable systems, the team began by zeroing in on a structural feature of the CRISPR Cas9 protein that binds to the enzyme's RNA guide. That is a key feature that has made Cas9 such a powerful tool.

"Being RNA-guided makes it relatively easy to reprogram, because we know how RNA binds to other DNA or other RNA," Zhang explains. His team searched hundreds of millions of biological proteins with known or predicted structures, looking for any that shared a similar domain. To find more distantly related proteins, they used an iterative process: from Cas9, they identified a protein called IS110, which had previously been shown by others to bind RNA. They then zeroed in on the structural features of IS110 that enable RNA binding and repeated their search.

At this point, the search had turned up so many distantly related proteins that the team turned to artificial intelligence to make sense of the list.

"When you are doing iterative, deep mining, the resulting hits can be so diverse that they are difficult to analyze using standard phylogenetic methods, which rely on conserved sequences," explains Guilhem Faure, a computational biologist in Zhang's lab.

With a protein large language model, the team was able to cluster the proteins they had found into groups according to their likely evolutionary relationships. One group set apart from the rest, and its members were particularly intriguing because they were encoded by genes with regularly spaced repetitive sequences reminiscent of an

essential component of CRISPR systems. These were the TIGR-Tas systems.

Zhang's team discovered over 20,000 different Tas proteins, mostly occurring in bacteria-infecting viruses. Sequences within each gene's repetitive region—its TIGR arrays—encode an RNA guide that interacts with the RNA-binding part of the protein. In some, the RNA-binding region is adjacent to a DNA-cutting part of the protein. Others appear to bind to other proteins, which suggests they might help direct those proteins to DNA targets.

Zhang and his team experimented with dozens of Tas proteins, demonstrating that some can be programmed to make targeted cuts to DNA in human cells. As they think about developing TIGR-Tas systems into programmable tools, the researchers are encouraged by features that could make those tools particularly flexible and precise.

They note that CRISPR systems can only be directed to segments of DNA that are flanked by short motifs known as PAMs (protospacer adjacent motifs). TIGR Tas proteins, in contrast, have no such requirement.

"This means theoretically, any site in the genome should be targetable," says scientific advisor Rhiannon Macrae.

The team's experiments also show that TIGR systems have what Faure calls a "dual-guide system," interacting with both strands of the DNA double helix to home in on their target sequences, which should ensure they act only where they are directed by their RNA guide. What's more, Tas proteins are compact—a quarter of the size of Cas9 on average—making them easier to deliver, which could overcome a major obstacle to therapeutic deployment of gene editing tools.

Excited by their discovery, Zhang's team is now investigating the natural role of TIGR systems in viruses as well as how they can be adapted for research or therapeutics. They have determined the molecular structure of one of the Tas proteins they found to work in human cells, and will use that information to guide their efforts to make it more efficient. Additionally, they note connections between TIGR-Tas systems and certain RNA-processing proteins in human cells.

"I think there's more there to study in terms of what some of those relationships may be, and it may help us better understand how these systems are used in humans," Zhang says.

More information: Guilhem Faure et al, TIGR-Tas: A family of modular RNA-guided DNA-targeting systems in prokaryotes and their viruses, *Science* (2025). DOI: 10.1126/science.adv9789

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