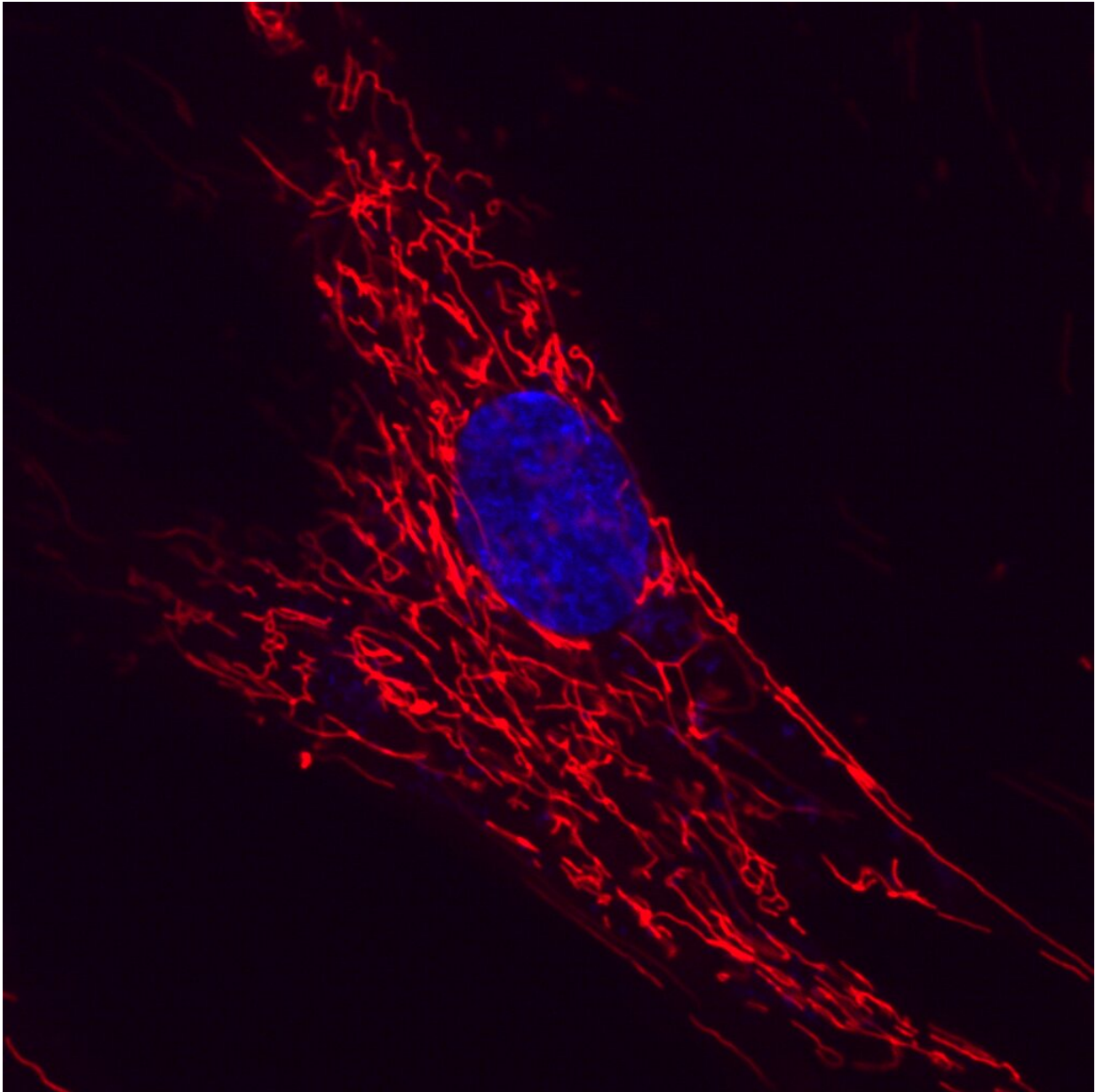


How to precisely edit mitochondrial DNA

July 8 2020



While precisely editing the DNA in a cell's nucleus (blue) has become easier

thanks to advances like CRISPR/Cas-9, it's been much harder to edit DNA in the mitochondria (red). Now, a new tool brings precision gene editing to mitochondrial DNA. Credit: Tslil Ast/Mootha Lab

Scientists can now precisely edit the genes inside mitochondria, the tiny energy factories inside of cells.

Over the past decade, gene editing has exploded in popularity. With CRISPR/Cas9 and related technologies, scientists can make targeted changes to DNA more easily than before. But while these state-of-the-art tools work in a cell's nucleus, where most genetic information is stored, some parts of cells have remained stubbornly out of reach for CRISPR.

Now, a new CRISPR-free tool brings that gene editing power to cells' second, smaller genome—the one in their mitochondria. It's the first precision gene editor for mitochondrial DNA, says Joseph Mougous, a Howard Hughes Medical Institute (HHMI) Investigator at the University of Washington.

The advance is the result of a cross-country collaboration between Mougous and two other HHMI Investigators—David Liu of Harvard University and the Broad Institute and Vamsi Mootha of Massachusetts General Hospital and the Broad Institute. The team, led by Mougous and Liu, reported its findings July 8, 2020 in the journal *Nature*.

Mutations in mitochondrial DNA can lead to a variety of rare and poorly understood diseases.

Until now, scientists studying these diseases in the lab could get rid of mutations by destroying the organelle's DNA. But they couldn't reliably correct a single mutation while leaving other mitochondria genes intact,

Liu says.

While this new tool is far from ready for use in humans, it'll make it easier for scientists to study those diseases, as well as basic mitochondrial biology, in animals, Mootha says. "This is a transformative technology for my field," he says. "It will now be possible to create mouse models of mitochondrial DNA disease—this has been super difficult until now."

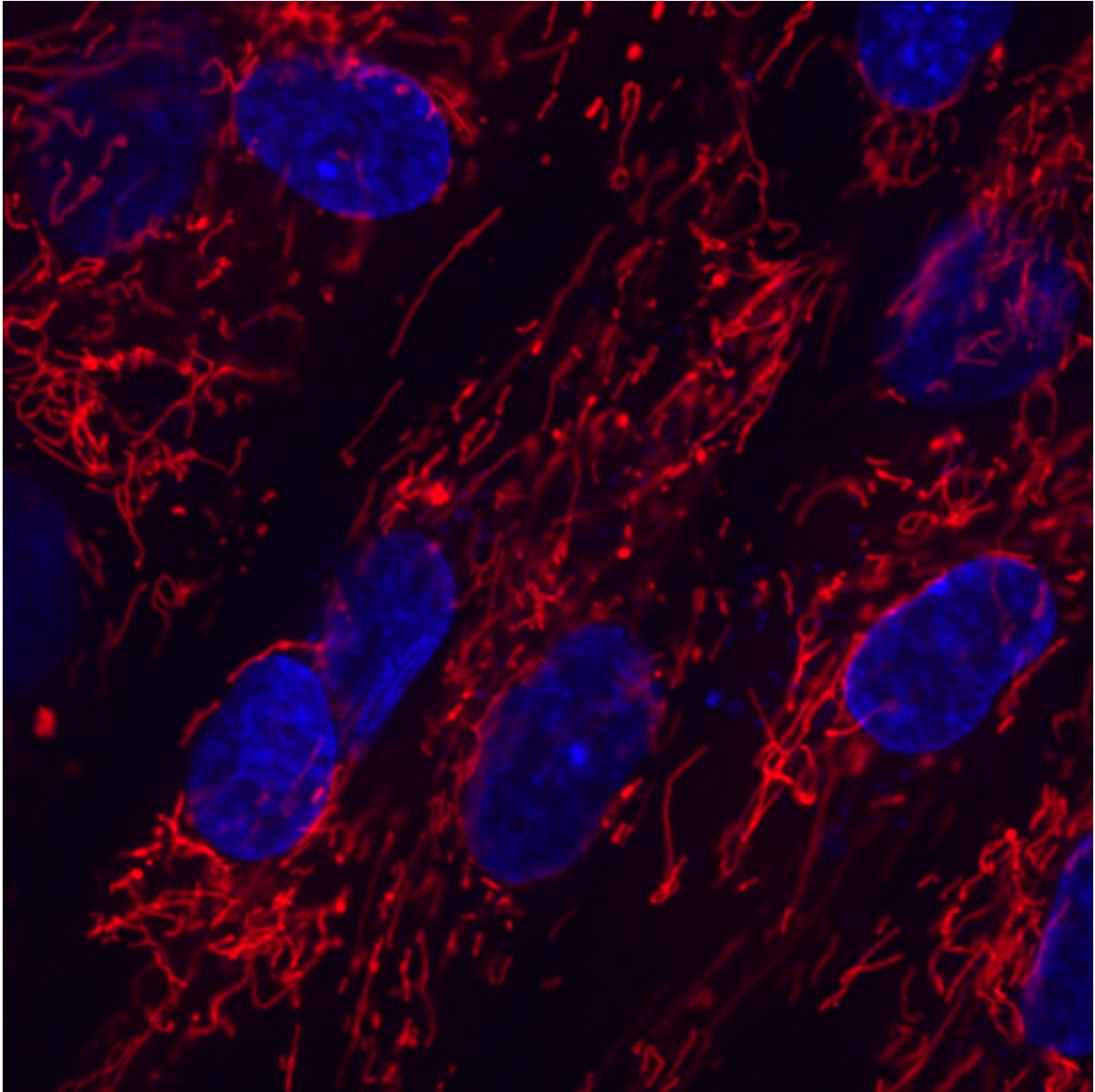
An unusual protein

Mougous didn't set out to create a gene editor. His lab studies bacterial warfare—specifically, the toxins that bacteria use to attack other bacteria.

Two years ago, Marcos de Moraes, a postdoc in Mougous's lab, was trying to understand how one of these toxins worked. But it was defying all his expectations. The toxin was a deaminase, a protein that can cause genetic mutations by removing nitrogen-containing pieces from DNA and RNA "letters."

Most deaminases target single strands of DNA, or RNA, which is naturally single-stranded. This deaminase was odd—it didn't appear to work on either. For months, de Moraes unsuccessfully tested the protein. Then one night, alone in the lab, he decided to try it out on something he didn't expect to work: double-stranded DNA.

DNA is usually found as a double helix, but testing the protein on it in this form "just didn't cross our minds," Mougous says. "It seemed that deaminases only worked on single-stranded DNA—end of story."



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But the unusual deaminase surprised them. It left the double-stranded

DNA in tatters, de Moraes says, weakening the DNA at every spot where it had edited a letter. "BOOM, we had this super-positive result!" he says. "It was probably the most exciting moment of my scientific career."

De Moraes and his colleagues spent the next few months confirming their initial findings. And then, sensing that the lab might be onto something useful for gene editing, Mougous reached out to Liu.

A new editor

Liu's lab was already pushing the boundaries of gene editing. His team had previously developed several precision tools for editing DNA in the cell nucleus, including "[base editors](#)" that can change individual letters. But mitochondrial gene editing had been tougher.

The famed CRISPR/Cas9 system relies on a small piece of "guide" RNA to lead the Cas9 enzyme to a specific spot in the genome, where it can snip both strands of DNA. Liu's team's base editors use the same approach. But CRISPR hasn't been able to edit mitochondrial DNA—because nobody has figured out how to transport guide RNA into mitochondria, Liu says.

A deaminase that worked directly on double-stranded DNA might be a workaround—no guide RNA required, the researchers realized. Liu took Mougous's initial call about the new deaminase during his morning commute and was so caught up in the conversation, he didn't hang up when he arrived at work.

Mougous's new molecule was exciting, but it was a naturally-occurring bacterial toxin, not a ready-made gene editor. Left unchecked, it would rampantly destroy DNA anywhere it could, just as de Moraes had shown in his early lab tests. To "tame the beast," as Liu describes it, he'd have

to find a way to prevent the deaminase from altering DNA until it got to just the right spot.

The solution: splitting the protein into two harmless halves. Liu's team, led by graduate student Beverly Mok, relied on 3-D imaging data from Mougous's lab to divide the protein into two pieces. Each piece did nothing on its own, but when reunited, they reconstituted the protein's full power. The team fused each deaminase half to customizable DNA-targeting proteins that did not require guide RNAs. Those proteins bound to specific stretches of DNA, bringing the two halves together. That let the deaminase regain function and work as a precision gene editor -but only once it was correctly positioned.

Liu's team used the technology to make precise changes to specific mitochondrial genes. Then, Mootha's lab, which focuses on mitochondrial biology, ran tests to see whether the edits had the intended effect.

"You could imagine that if you're introducing editing machinery into the mitochondria, you might accidentally cause some sort of a catastrophe," Mootha says. "But it was very clean." The entire mitochondrion functioned well, except for the one part the scientists intentionally edited, he explains.

This mitochondrial base editor is just the beginning, Mougous says. It can change one of the four DNA letters into another. He hopes to find more deaminases that he and Liu can develop into editors able to make other mitochondrial DNA alterations.

"One of the most fun aspects of this work has been the fact that our three labs came together organically," says Liu. "Not because anybody told us to get together and do something, but because that's where the science led us."

More information: A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing, *Nature*, [DOI: 10.1038/s41586-020-2477-4](https://doi.org/10.1038/s41586-020-2477-4) ,
www.nature.com/articles/s41586-020-2477-4

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