The enzyme that gives a powerful tool known as a “base editor” the ability to change DNA also has an off-target effect on RNA (above).

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Powerful CRISPR cousin accidentally mutates RNA while editing DNA target

By **Jon Cohen**Apr. 17, 2019 , 4:10 PM

When researchers first reported 3 years ago that they had created base editors, a version of the powerful genome-editing tool CRISPR, **excitement swirled** around their distinct powers to more subtly alter DNA compared with CRISPR itself. But the weaknesses of base editors have become increasingly apparent, and a new study shows they can also accidentally mutate the strands of RNA that help build proteins or perform other key cellular tasks. Researchers say this could complicate developing safe therapies with the technology and hamper other research applications.

Human diseases from sickle cell to Tay-Sachs are caused by a single mutation to one of the four DNA bases—adenine, guanine, cytosine, and thymine—and CRISPR has often had difficulty swapping out the bad actors. That’s in part because CRISPR cuts double-stranded DNA at targeted places and then relies on finicky cell repair mechanisms to do the heavy lifting of inserting a corrected DNA sequence for a mutation. Base editors, in contrast, chemically change one DNA base into another with enzymes called deaminases, which doesn’t require a cut or help from the cell.

Base editors, which adapt key components of CRISPR to reach targeted places in the genome, have been shown to have many **off-target effects on DNA**. But until now, its effects on RNA, which contains three of the same bases as DNA, had escaped scrutiny. So J. Keith Joung, a pathologist and molecular biologist at the Massachusetts General Hospital in Boston, led a team that put base editors into human liver and kidney cells. Their finding: **Deaminases can also alter RNA**, the group reports today in *Nature*.

Joung, a pioneering developer of base editors, was startled by the RNA changes, which had cytosines being converted to uracil, an RNA base that’s related to thymine. “When a postdoc first showed me the results and we saw tens of thousands of RNA cytosines being edited, I was like, ‘Wait a minute, what are we looking at here?’”

Jia Chen, who does genome editing research at ShanghaiTech University in China and was not involved in the new work, was not as surprised, noting that deaminases were originally described as having the ability to alter RNA. But he says the new work will push the field to solve the problem. “The finding will [lead to] developing novel base editors with higher editing precision,” Chen says.

Joung says his recent discovery of the old deaminase literature is what led his lab to do these experiments. And they’ve already engineered deaminases that substantially reduce the number of inadvertent RNA edits. “That was very encouraging to us,” Joung says. “We’re ultimately protein engineers, and we want to figure out if we can engineer the system to make the mutations go away.”

David Liu, a Harvard University chemist who created the first base editor and co-founded two companies based on the technology with Joung, notes that deaminases naturally edit cellular RNA, stressing that the biological consequences of such editing are unclear. He adds that his own lab’s studies of base editors have also found RNA off-target edits, but at far lower levels. The differences between their results, says Liu, likely have less to do with the amount of off-target RNA editing that takes place than the different way Joung’s group sorted its cells and analyzed the results.

Both Liu and Joung stress that their labs have found deaminases that work only on either DNA or RNA, which makes them confident that they can decouple the off-target effects seen with the current base editors. “Base editors are still incredibly powerful tools,” Joung says. “This is just another parameter we need to understand.”

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