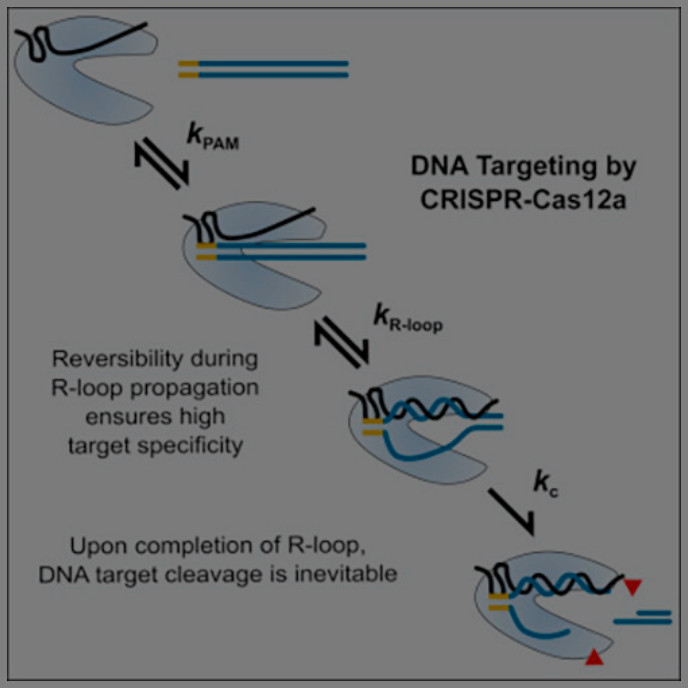
CRISPR-Cas12a More Precise Than CRISPR-Cas9

August 3, 2018



A quantitative kinetics study has dissected the reaction steps whereby Cas12a targets DNA. This study explains the DNA cleavage patterns measured in vivo and the observations of greater reported target specificity for Cas12a than for the Cas9 nuclease. [Molecular Cell]

In CRISPR tool development, Cas9 got there firstest, but not necessarily

recognize and destroy viral infections. As these CRISPR systems came to be adapted for human purposes, they started to show just how varied they were, particularly with respect to their CRISPR-associated (Cas) enzymes, which follow guide RNA and cut DNA where guide RNA specifies.

Alas, none of the Cas enzymes is perfect, not even the popular Cas9, the first Cas enzyme discovered by researchers. Because Cas enzymes occasionally fail to cut DNA in the right places, or even cut at all, they worry developers, who want to modify genomes with surgical precision, especially in therapeutic applications.

"The overall goal is to find the best enzyme that nature gave us and then make it better still, rather than taking the first one that was discovered through historical accident," says Ilya Finkelstein, Ph.D., an assistant professor of molecular biosciences at the UT-Austin. Dr. Finkelstein was part of a team of scientists that decided to clarify where Cas9 stood with respect to Cas12a.

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Molecular Cell. According to this paper, Cas12 is a more precise gene modifying instrument than Cas9.

"We show that Cas12a binds DNA tightly in two kinetically separable steps," the article's authors wrote. "Protospacer-adjacent motif (PAM) recognition is followed by rate-limiting R-loop propagation, leading to inevitable DNA cleavage of both strands.

"Despite functionally irreversible binding, Cas12a discriminates strongly against mismatches along most of the DNA target sequence. This result implies substantial reversibility during R-loop formation—a late transition state—and defies common descriptions of a 'seed' region."

Essentially, the UT-Austin team found that Cas12a is choosier because it binds like Velcro to a genomic target, whereas Cas9 binds to its target more like super glue. Each enzyme carries a short string of genetic code written in RNA that matches a target string of genetic code written in the DNA of a virus. When it bumps into some DNA, the enzyme starts trying to bind to it by forming base pairs—starting at one end and working its way along,

testing to see how well each letter on one side (the DNA) matches the adjacent letter on the other side (the RNA).

For Cas9, each base pair sticks together tightly, like a dab of super glue. If th
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For Cas12a, it's more like a Velcro strap. At each point along the way, the bonds are relatively weak. It takes a good match all along the strip for the two sides to hold together long enough to make an edit. That makes it much more likely that it will edit only the intended part of the genome.

"It makes the process of base-pair formation more reversible," says Rick Russell, Ph.D., professor of molecular biosciences and senior author of the current study. "In other words, Cas12a does a better job of checking each base pair before moving on to the next one. After seven or eight letters, Cas9 stops checking, whereas Cas12a keeps on checking out to about 18 letters."

The researchers said that Cas12a still isn't perfect, but the study also suggests ways that Cas12a can be improved further, perhaps one day realizing the dream of creating a "precision scalpel," an essentially error-proof gene-editing tool.

"On the whole, Cas12a is better, but there were some areas where Cas12a was still surprisingly blind to some mispairing between its RNA and the

genomic target," adds Dr. Finkelstein. "So, what our work does is show a clear path forward for improving Cas12a further."

The researchers are currently using these insights in a follow-on project d

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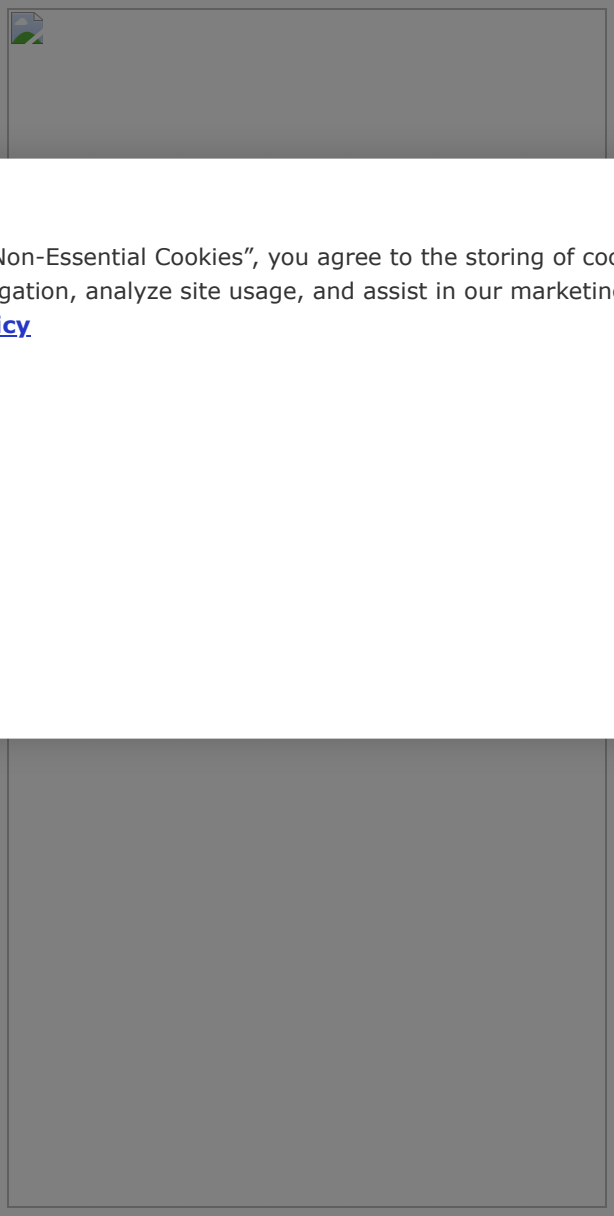

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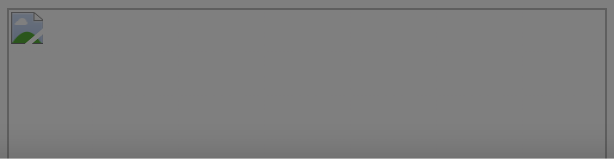

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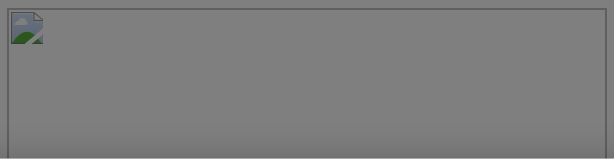

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