New Cas9 variant makes genome editing even more precise

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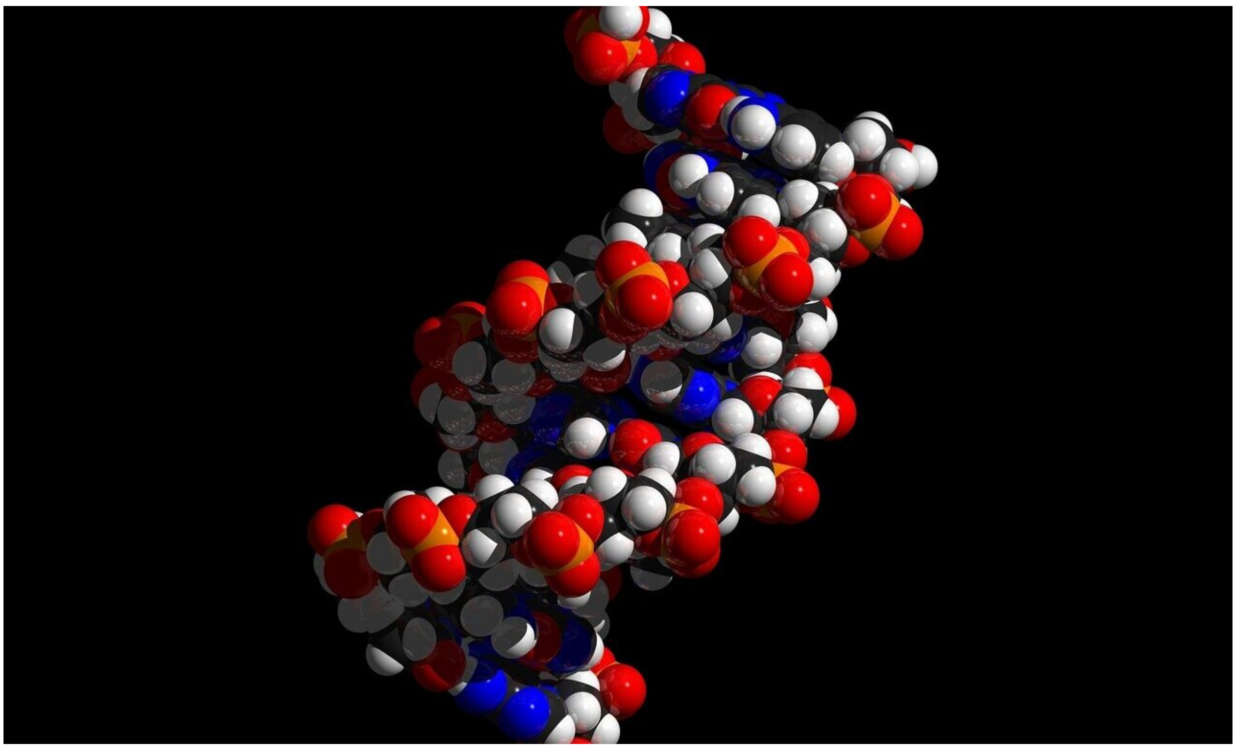

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CRISPR-Cas9 has revolutionized the field of genetics by its ability to cut DNA at defined target sites. Researchers are using the Cas9 enzyme to specifically switch off genes, or insert new DNA fragments into the genome. But no matter how specific the Cas9 enzyme is—sometimes it cuts where it shouldn't. Scientists at the Max Planck Unit for the Science of Pathogens in Berlin and the Faculty of Medicine of the Martin Luther University Halle-Wittenberg now report a Cas9 variant that increases the specificity of genome editing.

In order for Cas9 to cut a DNA target, it needs to be directed to the target site by what is called a guide RNA. The guide RNA contains the complementary sequence to the DNA target site, working like a ZIP Code to guide Cas9 to its target. "Sometimes, however, Cas9 can also cut DNA sequences that are very similar to the actual target, known as off-targets," explains Emmanuelle Charpentier, director of the Max Planck Unit for the Science of Pathogens.

This undesired activity of CRISPR-Cas9 can lead to inaccuracies in genome editing. "An unintended cut at the wrong place in the human genome can have profound consequences. That is why we need a more specific system," says Michael Böttcher, Assistant Professor at the Medical Faculty of the Martin Luther University.

Scientists are therefore trying to optimize Cas9 specificity using different approaches. In the current study, the team of researchers from Berlin and Halle focused on an evolutionarily conserved domain of Cas9, known as bridge helix.

Amino acids form stable loop

The researchers found that the bridge helix plays a critical role in the mechanism by which Cas9 interacts with its guide RNA and DNA target site. They identified a group of amino acid residues that make contact with the phosphate backbone of the guide RNA, thereby facilitating the formation of a stable loop, which is essential for the activity of Cas9. In such a loop, the Cas9-bound guide RNA pairs with the complementary strand of the DNA target sequence while displacing the second DNA strand, thereby enabling Cas9 to cut both DNA strands.

The researchers generated new Cas9 variants by changing these amino acid residues and found that several variants cut much less frequently at off-target sites than the original Cas9 enzyme. They further show that one of the identified variants, called R63A/Q768A, increased the gene editing specificity of Cas9 also in human cells. "Our results provide a new basis for further optimization of CRISPR-Cas9. They demonstrate the need to gain more knowledge about the biochemistry of CRISPR-Cas systems to further improve them," says Charpentier.

More information: Majda Bratovič et al. Bridge helix arginines play a critical role in Cas9 sensitivity to mismatches, *Nature Chemical Biology* (2020). DOI: 10.1038/s41589-020-0490-4

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