New Cas9 variant makes genome editing even more precise

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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.

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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.

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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 Bratović et al. Bridge helix arginines play a critical role in Cas9 sensitivity

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