How molecular scissors cut in the right place

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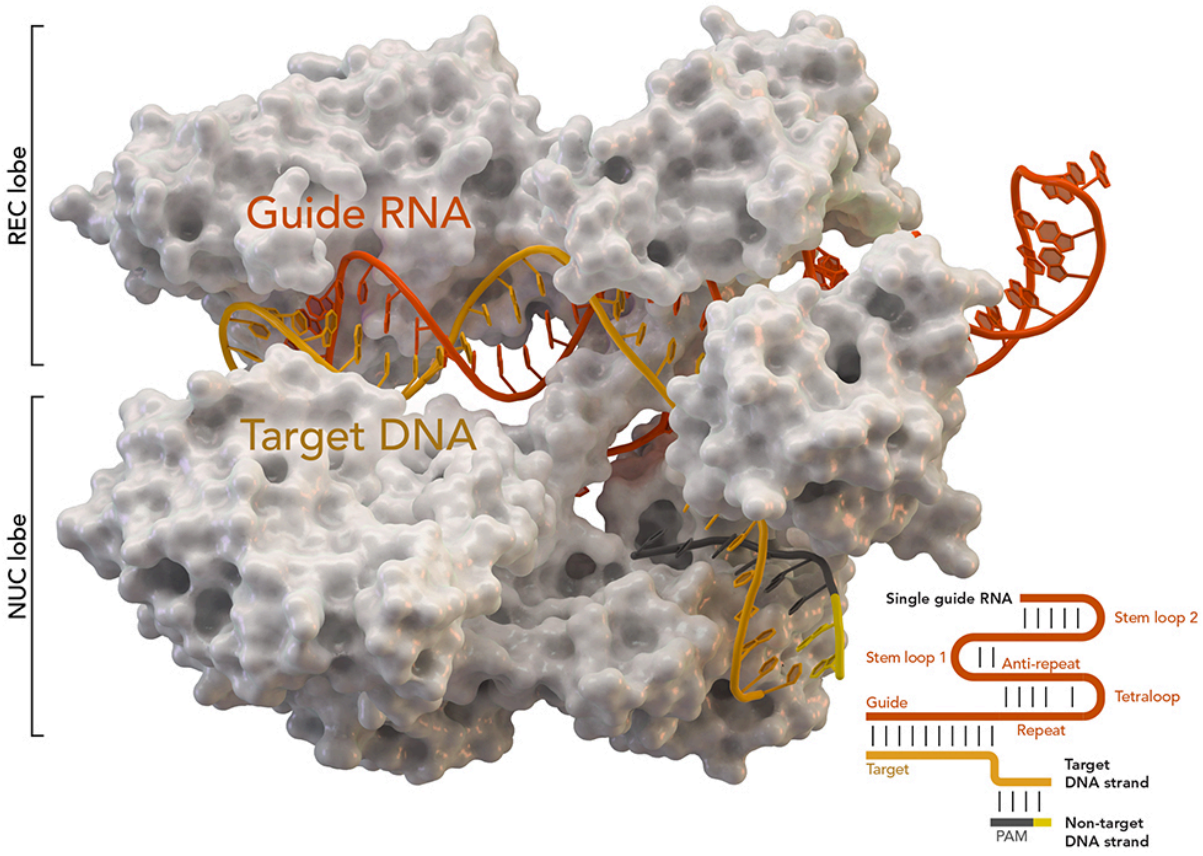

CRISPR-associated protein Cas9 (white) from Staphylococcus aureus based on Protein Database ID 5AXW. Credit: Thomas Splettstoesser (Wikipedia, CC BY-SA 4.0)

A research group at Uppsala University has found out how CRISPR-Cas9—also known as 'molecular scissors'—can search the genome for a

specific DNA sequence. Cas9 already has many applications in biotechnology and is also expected to revolutionise medicine. The new research findings show how Cas9 can be improved to make the molecular scissors faster and more reliable. The study is being published in *Science*.

In less than a decade, CRISPR-Cas9 has revolutionised biological research. Cas9 makes it possible, for specific purposes, to correct or modify ('edit') essentially any DNA sequence. The hope is that the genetic scissors will also enable genetic diseases to be cured and prevented.

The exciting aspect of Cas9 is that the molecule can be programmed with a piece of artificial genetic code, which can then be made to seek out the corresponding sequence in the genome. A research group at Uppsala University has now discovered how Cas9 finds the right sequence.

'Most proteins that search DNA code can recognise one specific sequence merely by sensing the outside of the DNA double helix. Cas9 can search for an arbitrary code, but to determine whether it is in the right place the molecule has to open the double DNA helix and compare the sequence with the programmed code. The incredible thing is that it can still search the entire genome without using any energy,' says Johan Elf, who is in charge of the study.

The researchers have developed two new methods to measure how long Cas9 takes to find its target sequence. The first method showed that it takes as long as six hours for Cas9 to search a bacterium, i.e. through four million base pairs. This somewhat unlikely result was also verifiable by means of the second, independent technique. The time found also tallies with the number of milliseconds Cas9 has available for testing every position, which the researchers were able to measure by following

labelled Cas9 molecules in real time.

'The results show that the price Cas9 pays for its flexibility is time. To find the target faster, more Cas9 molecules searching for the same DNA sequence are needed,' says Johan Elf.

The very high concentrations of Cas9 that are necessary for finding the right sequence within a reasonable time frame can pose severe problems for the cells that scientists try to alternate. But since the nature of the search process is now understood, an important clue as to how the system can be improved has been found. By sacrificing a portion of Cas9's flexibility, it would be possible to design genetic scissors that are still sufficiently versatile to edit various genes but simultaneously fast enough to be medically usable.

'The results have given us clues on how we might achieve that kind of solution,' Elf says. 'The key is in what are known as the "PAM sequences", which determine where and how often Cas9 opens up the DNA double helix. Molecular scissors that do not need to open the helix as many times to find their target are not only faster but would also reduce the risk of side-effects."

More information: "Kinetics of dCas9 target search in Escherichia coli" *Science* (2017). science.sciencemag.org/cgi/doi … 1126/science.aah7084

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