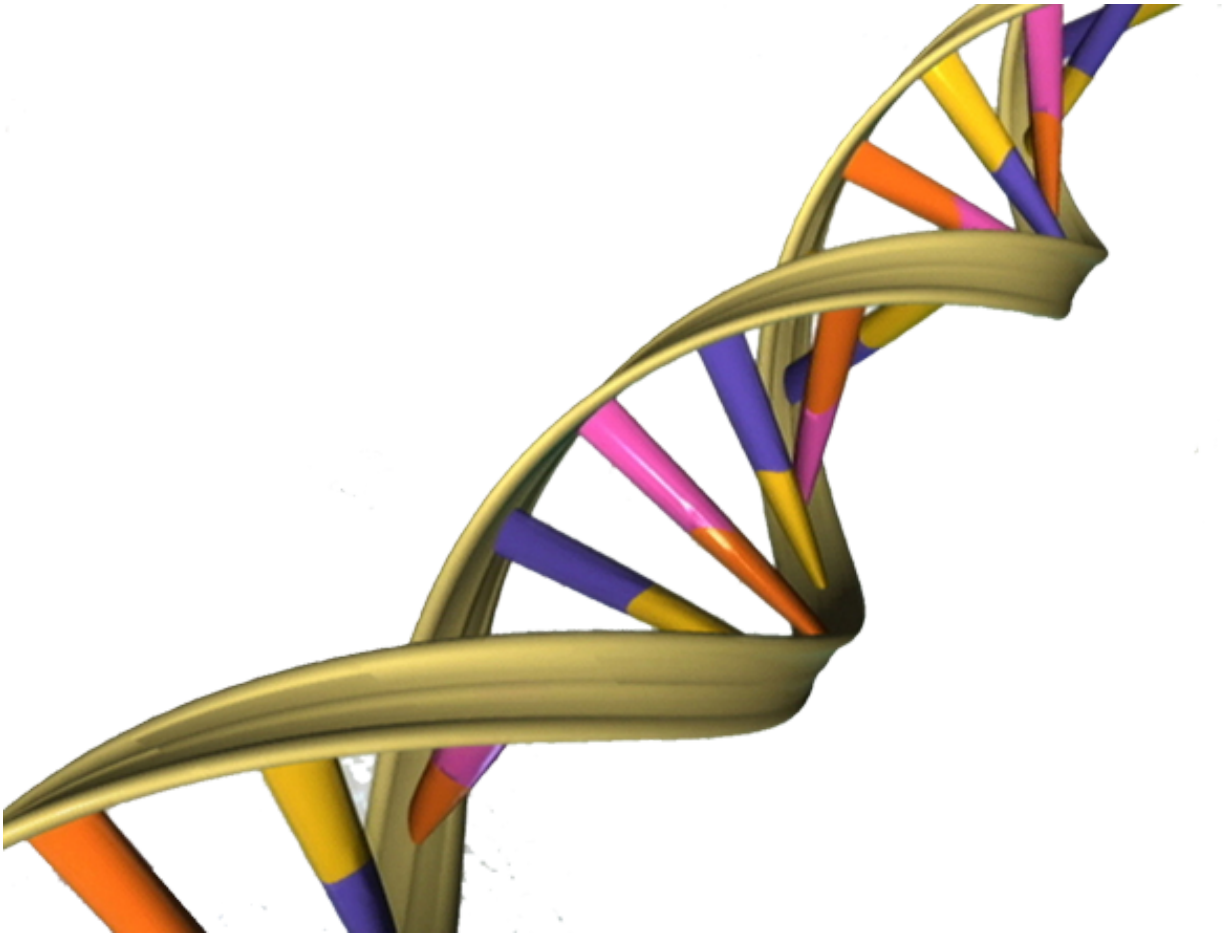
# Cpf1: CRISPR-enzyme scissors cutting both RNA and DNA

April 21 2016



A depiction of the double helical structure of DNA. Its four coding units (A, T, C, G) are color-coded in pink, orange, purple and yellow. Credit: NHGRI

Only a few years after its discovery, it is difficult to conceive of genetics without the CRISPR-Cas9 enzyme scissors, which allow for a very simple, versatile and reliable modification of DNA of various organisms. Since its discovery, scientists throughout the world have been working on ways of further improving or adjusting the CRISPR-Cas9 system to their specific needs.

Researchers from the Max Planck Institute for Infection Biology in Berlin, the Umeå University in Sweden and the Helmholtz Centre for Infection Research in Braunschweig have now discovered a feature of the CRISPR-associated protein Cpf1 that has previously not been observed in this family of enzymes: Cpf1 exhibits dual, RNA and DNA, cleavage activity. In contrast to CRISPR-Cas9, Cpf1 is able to process the pre-crRNA on its own, and then using the processed RNA to specifically target and cut DNA. Not requiring a host derived RNase and the tracrRNA makes this the most minimalistic CRISPR immune system known to date. The mechanism of combining two separate catalytic moieties in one allows for possible new avenues for sequence specific genome engineering, most importantly facilitation of targeting multiple sites at once, the so-called multiplexing.

CRISPR-Cas is part of the immune system of bacteria and is used to fight viruses. In the CRISPR-Cas9 system, the enzyme Cas9 cuts the virus DNA at a location specified by an RNA molecule – known as CRISPR RNA (crRNA) in complex with another RNA, the so-called tracrRNA. This puts the pathogens out of action.

In 2011, Emmanuelle Charpentier and her co-workers described that the system consists of two RNAs forming a duplex (tracrRNA and pre-crRNA), with tracrRNA maturing pre-crRNA to crRNA, in the presence of the protein Cas9 (formerly named Csn1). A year later Emmanuelle Charpentier and colleagues demonstrated that tracrRNA and crRNA together, be it in form of the duplex of two guide RNAs or a fused single

guide RNA, are required to specifically guide the Cas9 enzyme to the matching target DNA sequence.

Since then, CRISPR-Cas9 has taken laboratories by storm. Both scientists and clinicians have great hopes for it: the latter aims to use the enzyme scissors to cure severe genetic diseases.

"Although the workings of CRISPR-Cas9 sound simple, the details of the mechanisms involved are rather subtle," says Charpentier, Director at the Max Planck Institute for Infection Biology. Before the crRNA molecule can show the Cas9 protein the cutting point, it must be transformed into its final form itself: RNA-cleaving proteins are needed so that a functioning crRNA arises. One of these is RNase III. In 2011, Charpentier discovered that this enzyme is involved in the crRNA maturation process along with tracrRNA.

## A minimalistic CRISPR-system

The researchers have now discovered that the immune defence mechanism of some bacteria is simpler in structure than CRISPR-Cas9. In addition to Cas9, these bacteria use the enzyme Cpf1 for cleaving foreign DNA. The results now show that Cpf1 can cut both RNA and DNA. Cpf1 first removes sections of the crRNA and thereby assists the maturation. Additional maturation enzymes like RNase III are not required. The mature RNA-molecule then guides Cpf1 to its target section on the DNA.

Cpf1 thus has a dual function: it enables the functioning of crRNA and then cleaves the DNA in a sequence specific manner. In addition, unlike Cas9, Cpf1 is not depending on the help of a tracrRNA molecule to reach its destination. Consequently, it is even simpler in structure than CRISPR-Cas9. "CRISPR-Cpf1 is a plug-and-play system with no additional component needed. In contrast, CRISPR-Cas9 needs in its

natural setting an assistant to activate the system," explains Charpentier.

"If the CRISPR-Cpf1 system provides any tangible added value over the CRISPR-Cas9 system when it comes to eukaryotic gene editing remains to be elucidated. However, it is stunning to see how evolution has succeeded to yield a dramatically minimalistic but effective immune system to fight invading viruses", says Charpentier. "There may be more such systems to be found in nature in the future, the search for them is already in full swing."

**More information:** Ines Fonfara et al. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA, *Nature* (2016). DOI: 10.1038/nature17945

Provided by Max Planck Society