Researchers show that nucleosomes can inhibit CRISPR-Cas9 cleavage efficiency

11 September 2018, by Bob Yirka

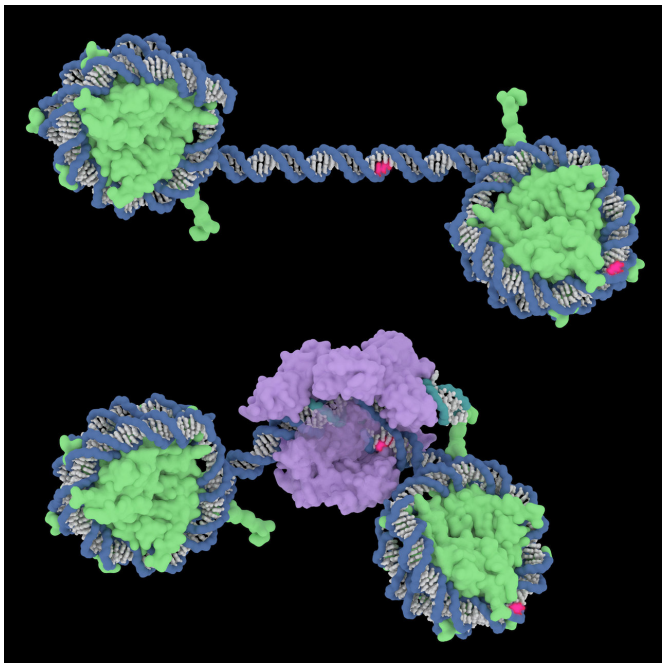

Illustration of Cas9 binding to DNA. The top figure shows two nucleosomes surrounding a nucleosome-free stretch of DNA. Hypothetical PAM sites for Cas9 targets in the central region and the right-hand nucleosome are shown in red. The lower figure shows Cas9 (with sgRNA) bound to the central target, with the PAM and flanking DNA held deep in the protein cleft. The PAM in the nucleosome could not be accessed without dissociating the DNA from the histone core. DNA backbones are blue; the RNA backbone is teal; DNA and RNA bases are white, except for the PAM; histones are green; Cas9 is purple. Credit: Janet Iwasa (University of Utah, Salt Lake City).

A team of researchers at the University of Utah has found that nucleosomes can inhibit CRISPR/Cas9 cleavage efficiency. In their paper published in *Proceedings of the National Academy of Sciences*, the group describes testing the gene editing technique on yeast samples and what they found.

The gene editing technique CRISPR-Cas 9 uses guide RNA to find and snip out segments of DNA—but what happens when the targeted segment is part of a nucleosome? Prior research has suggested that in such instances, it is likely that cleavage efficiency would suffer. In this new effort, the researchers have carried out an in vivo test of such instances and found that prior research results were correct—using CRISPR-Cas 9 on nucleosomes may not work very well.

DNA strands are tiny, but really long—approximately six feet long if stretched out. Because of that, cells have mechanisms for packing DNA into a cell nucleus. That mechanism involves rolling the strands into bunches around a given protein. Such rolled bunches are known as nucleosomes. Logic suggests that a technique for editing a strand of DNA might encounter difficulty because of accessibility issues. Other researchers have considered the possibility of such problems, but have studied them in test tubes or did their work on known strands that were not part of nucleosomes. In this new effort, the researchers sought to find out once and for all if CRISPR-Cas9 would work just as well editing strands that are part of nucleosomes in living tissue as it does on strands that are not.

The work involved CRISPR-Cas9 gene editing using different guide RNAs in living yeast, which allowed for editing different targets. Some of the targets were in nucleosomes while others were not.

The researchers report that cleavage efficiency was much lower in nucleosomes than in non-nucleosome areas. But they also found something else—when testing the gene editing technique called zinc fingers the same way, they found no such difference. The group suggests that in the future, gene editing efforts should start with nucleosome position maps to improve efficiency.

More information: Robert M. Yarrington et al. Nucleosomes inhibit target cleavage by CRISPR-

Cas9 in vivo, *Proceedings of the National Academy of Sciences* (2018). DOI: 10.1073/pnas.1810062115

Abstract
Genome editing with CRISPR-Cas nucleases has been applied successfully to a wide range of cells and organisms. There is, however, considerable variation in the efficiency of cleavage and outcomes at different genomic targets, even within the same cell type. Some of this variability is likely due to the inherent quality of the interaction between the guide RNA and the target sequence, but some may also reflect the relative accessibility of the target. We investigated the influence of chromatin structure, particularly the presence or absence of nucleosomes, on cleavage by the Streptococcus pyogenes Cas9 protein. At multiple target sequences in two promoters in the yeast genome, we find that Cas9 cleavage is strongly inhibited when the DNA target is within a nucleosome. This inhibition is relieved when nucleosomes are depleted. Remarkably, the same is not true of zinc-finger nucleases (ZFNs), which cleave equally well at nucleosome-occupied and nucleosome-depleted sites. These results have implications for the choice of specific targets for genome editing, both in research and in clinical and other practical applications.

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