Dually noted: New CRISPR-Cas9 strategy edits genes two ways

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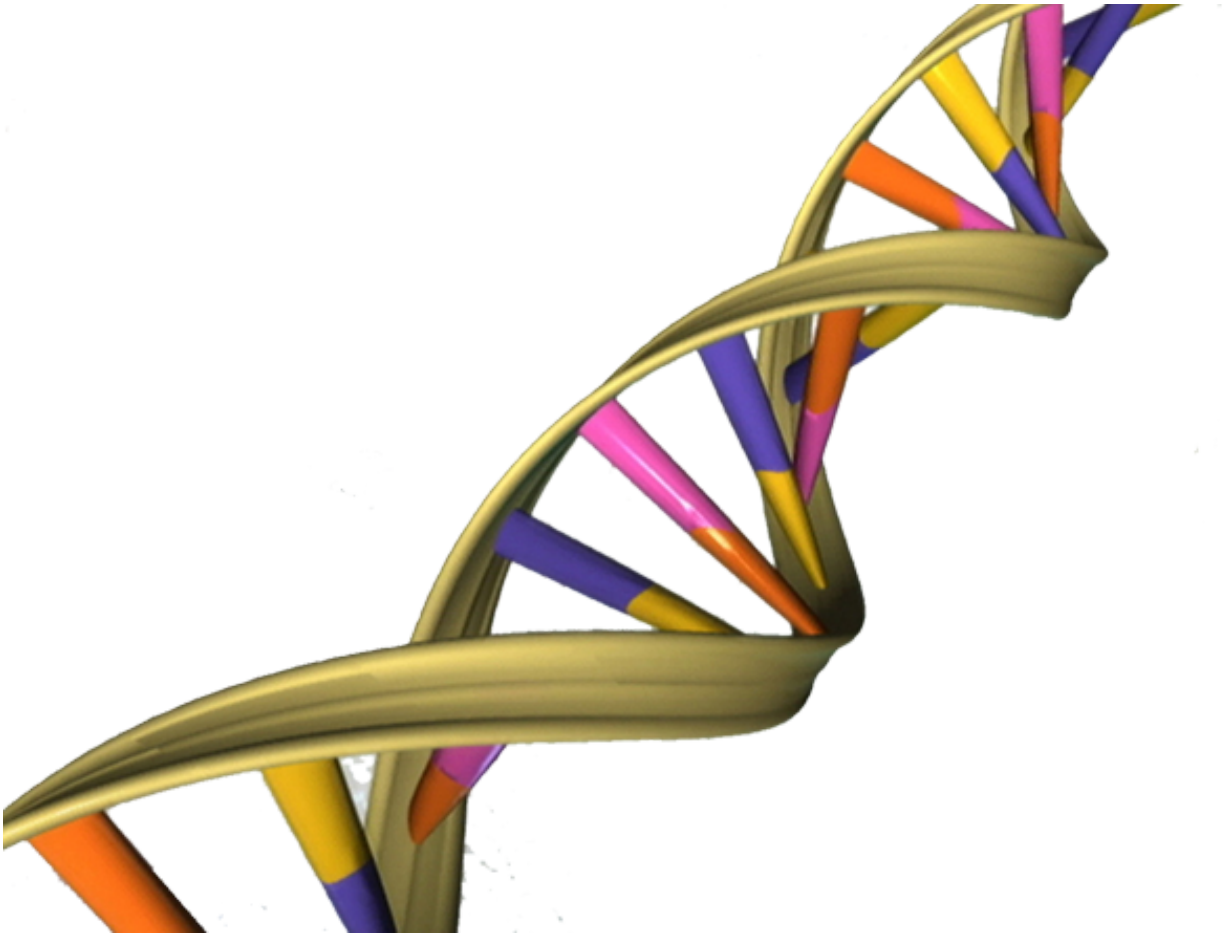

A depiction of the double helical structure of DNA. Its four coding units (A, T, C, G) are color-coded in pink, orange, purple and yellow. Credit: NHGRI

The CRISPR-Cas9 system has been in the limelight mainly as a revolutionary genome engineering tool used to modify specific gene sequences within the vast sea of an organism's DNA. Cas9, a naturally occurring protein in the immune system of certain bacteria, acts like a pair of molecular scissors to precisely cut or edit specific sections of DNA. More recently, however, scientists have also begun to use CRISPR-Cas9 variants as gene regulation tools to reversibly turn genes on or off at whim.

Both of these tasks, genome engineering and gene regulation, are initiated with a common step: the Cas9 protein is recruited to targeted genes by the so-called matching sequences of "guide RNA" that help Cas9 latch on to specific sequences of DNA in a given genome. But until now, genome engineering and gene regulation required different variants of the Cas9 protein; while the former task hinges on Cas9's innate DNA-cleaving activity, the latter has been achieved by engineered Cas9 variants that have had their DNA-cleaving "fangs" removed, but still retain their ability to latch onto a specific genomic target. These latter Cas9 variants are commonly fused with proteins that regulate gene expression.

Now, using a new approach developed by researchers led by George Church, Ph.D., of Harvard and Ron Weiss, Ph.D., of the Massachusetts Institute of Technology, both tasks can be achieved using one type of Cas9, allowing scientists to increase the complexity of gene editing functions and their overall control of genes. The method opens up unexpected possibilities for understanding diseases and drug mechanisms. The study's findings are reported in the September 7 issue of *Nature Methods*.

Church is Core Faculty member at Harvard's Wyss Institute for Biologically Inspired Engineering, Robert Winthrop Professor of Genetics at Harvard Medical School and Professor of Health Sciences

and Technology at Harvard and MIT, and Weiss is Professor of Biological Engineering and also Professor of Electrical Engineering and Computer Science at MIT. Their multi-institutional team has introduced the clever new kit that allows the innate Cas9 protein from Streptococcus pyogenes to cleave certain genes while simultaneously regulating the expression of others through engineering the guide RNA. James Collins, Ph.D., Wyss Institute Core Faculty member and the Termeer Professor of Medical Engineering & Science and Professor of Biological Engineering at MIT, is also a co-investigator and a co-author on the study.

Key to their strategy, the team discovered that the length of the guide RNA sequence plays a critical role in determining whether or not Cas9 will solely bind to DNA or if it will excise it as well.

"We decided to systematically test why it was that truncating guides too much caused Cas9 to no longer cut the intended genomic site," said Alejandro Chavez, Postdoctoral Fellow at the Wyss Institute. Chavez, who is advised by both Church and Collins at the Wyss, is a co-first author on the study together with Samira Kiani, Postdoctoral Associate in Weiss' MIT lab.

The Wyss and MIT team confirmed in human cells that shorter guide RNAs indeed no longer allowed Cas9 to cut a targeted gene. To their surprise, however, the shorter guide RNAs did not prevent Cas9 from efficiently binding to that target, opening up the possibility for scientists to attach gene regulation proteins to Cas9 for delivery to specific genes.

"By using our uncovered guide RNA principles, we can now for the first time toggle a single protein to gain direct control over both, gene sequences and gene expression, and turn almost any DNA sequence into a regulatory sequence to further bend the cell to our will. We envision future uses for the technology that can help decipher the tangled web of

interactions underlying for example cancer drug resistance and stem cell differentiation, or design advanced synthetic gene circuitries," said Church.

"This new functionality will improve our ability to decipher the complex relationships between interdependent genes responsible for many diseases," said Marcelle Tuttle, a Research Fellow at the Wyss Institute and co-author on the study.

The findings could also be used in large scale metabolic production of chemicals and fuels using genetically engineered bacteria - such as common E. coli - while safeguarding the "microbial workers" from infection by other microbes and pathogens.

"Cas9 has emerged as a revolutionary tool allowing us to conquer new biomedical and industrial territory. This team's findings harness yet another level of control and versatility in gene editing and demonstrate the Wyss Institute's continued efforts in advancing the use of the CRISPR-Cas9 system towards key applications," said Wyss Institute Founding Director Donald Ingber, M.D., Ph.D., who is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital and Professor of Bioengineering at the Harvard John A. Paulson School of Engineering and Applied Sciences.

More information: Cas9 gRNA engineering for genome editing, activation and repression, DOI: 10.1038/nmeth.3580

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