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Scientists crack the CRISPR code for precise human genome editing

December 13 2018

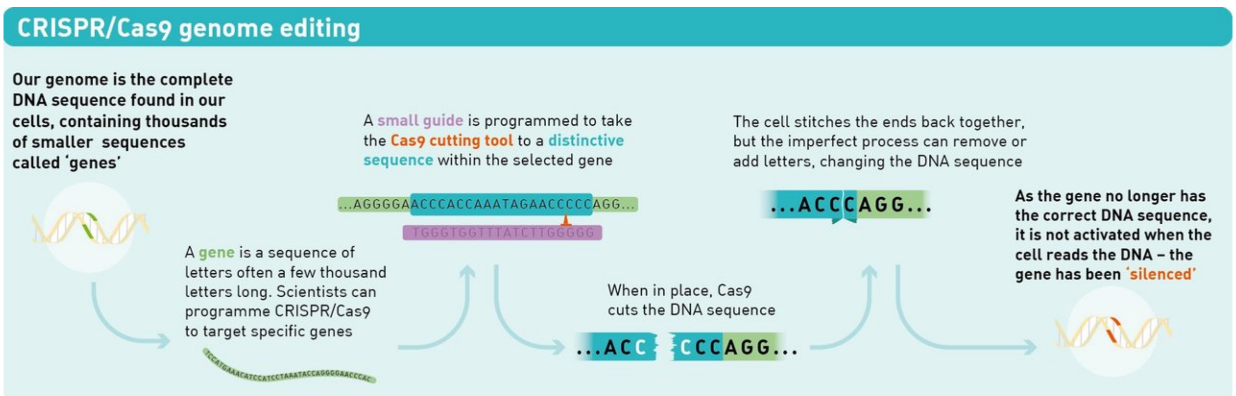

Illustration of the CRISPR/Cas9 genome editing system. Credit: Nigel Hawtin for the Francis Crick Institute

Scientists at the Francis Crick Institute have discovered a set of simple rules that determine the precision of CRISPR/Cas9 genome editing in human cells. These rules, published in *Molecular Cell*, could help to improve the efficiency and safety of genome editing in both the lab and the clinic.

Despite the wide use of the CRISPR system, rational application of the technology has been hindered by the assumption that the outcome of genome editing is unpredictable, resulting in random deletions or insertions of DNA regions at the target site.

Before CRISPR can be safely applied in the clinic, scientists need to make sure that they can reliably predict precisely how DNA will be modified.

"Until now, editing genes with CRISPR has involved a lot of guesswork, frustration and trial and error," says Crick group leader Paola Scaffidi, who led the study. "The effects of CRISPR were thought to be unpredictable and seemingly random, but by analysing hundreds of edits we were shocked to find that there are actually simple, predictable patterns behind it all. This will fundamentally change the way we use CRISPR, allowing us to study gene function with greater precision and significantly accelerating our science."

By examining the effects of CRISPR genome editing at 1491 target sites across 450 genes in human cells, the team have discovered that the outcomes can be predicted based on simple rules. These rules mainly depend on one genetic 'letter' occupying a particular position in the region recognized by the 'guide RNA' to direct the molecular scissors, Cas9 .

Guide RNAs are synthetic molecules made up of around 20 genetic letters (A,T,C,G), designed to bind to a specific section of DNA in the target gene. Each genetic letter has a complementary partner—A binds to T and C binds to G—which stick together a bit like Velcro. The guide RNA is like the 'hook' side of Velcro, designed to stick to the 'loop' side on the target gene.

Guided by the RNA molecule, the Cas-9 enzyme scans along the genome until it finds the region of interest. When the RNA guide matches the correct DNA sequence, it sticks like Velcro and Cas9 cuts through the DNA. The DNA is broken three letters from the end of the target sequence, and bits of genetic code are then inserted or deleted, seemingly haphazardly, when the cell attempts to repair the break.

In this study, the researchers found that the outcome of a particular gene edit depends on the fourth letter from the end of the RNA guide, adjacent to the cutting site. The team discovered that if this letter is an A or a T, there will be a very precise genetic insertion; a C will lead to a relatively precise deletion and a G will lead to many imprecise deletions. Thus, simply avoiding sites containing a G makes genome editing much more predictable.

"We were amazed to discover that the rules that determine the outcome of CRISPR human genome editing are so simple," says Dr. Anob Chakrabarti, Wellcome Trust clinical Ph.D. fellow in the Crick's Bioinformatics and Computational Biology lab and joint-first author of the study. "By bearing these rules in mind when designing our guide RNAs, we can maximise the chances of getting the desired outcome of a specific gene edit—which is particularly important in a clinical context."

The team also discovered that how 'open' or 'closed' the target DNA is also affects the outcome of gene editing. Adding compounds that force DNA to open up—allowing Cas9 to scan the genome—led to more efficient editing, which could help when modifications need to be introduced in particularly closed genes.

"The good news is that regardless of the tissue of origin—which influences the degree of DNA 'openness' at specific genes—target regions containing an A or T at the key position show common editing," says Paola. "This means that, if we carefully select the target DNA, we can be pretty confident that we'll see the same effect in different tissues."

Josep Monserrat, Crick Ph.D. student in the Cancer Epigenetics lab and joint-first author of the study, says: "We hadn't previously appreciated the significance of DNA openness in determining the efficiency of CRISPR genome editing. This could be another factor to consider when aiming to edit a gene in a specific way. We are excited to observe that distinct cell types share common editing at precise target regions, and hope translation of our findings will be beneficial across disciplines."

More information: *Molecular Cell* (2018). DOI: 10.1016/j.molcel.2018.11.031

Provided by The Francis Crick Institute