Making CRISPR-Cas9 a more powerful tool for gene editing

Sojung Kim
Taegeun Bae
Jaewoong Hwang
Jin-Soo Kim

Video Abstract

Keywords: CRISPR-Cas Cas9 Off-target effects Hammerhead Ribozyme SpCas9 variants gene editing genome editing plant cells agronomic editing protein engineering u3 promoter u6 promoter mismatch guide RNA tRNA RNase P RNase Z Institute for Basic Science Chinese Academy of Sciences

Posted Date: September 20th, 2019

DOI: <https://doi.org/10.21203/rs.2.15063/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

CRISPR-Cas9 genome editing has revolutionized biomedicine by enabling so-called genetic surgery on organisms ranging from single-cell microbes to sheep. Use of the technique, however, has been hampered by its low specificity, which can damage cells through unintended changes to DNA. New versions of the system have been engineered to avoid these off-target effects, but this has come at the cost of efficiency. Now, two separate teams have devised complementary ways to boost efficiency without sacrificing specificity, creating new promise for the field. The CRISPR-Cas9 system uses a protein called Cas9 and a guide RNA to make targeted breaks in DNA. When the guide RNA pairs with matching DNA, Cas9 cuts the DNA. The cut is then repaired by cellular machinery, and during this process, small pieces of DNA can be added or removed. One risk is that the system sometimes binds regions that aren't exact matches, potentially causing unplanned changes. Cas9 variants have been engineered to prevent this, but they don't cut DNA as efficiently as the original system. One barrier slowing them down is the presence of an extra nucleotide at one end of the guide RNA. When scientists use CRISPR-Cas9 to edit genes, they must deliver both the protein and the RNA to a cell. But the process used to make the RNA adds this extra nucleotide. The teams restored the system's efficiency by developing unique ways to address it. The first group linked an enzyme named Hammerhead to the guide RNA. Hammerhead cuts RNA at specific sites, and the team took advantage of this to remove the extra nucleotide to complement the target DNA. Adding the altered guide RNA to human cells with engineered Cas9 produced targeted cuts at high frequency. The second group used cellular machinery to remove the extra nucleotide. They combined the guide RNA with a tRNA sequence that, when recognized, is enzymatically removed by the cell. The researchers designed the guide RNA so that when the tRNA sequence was removed, the extra nucleotide went along with it. When the cut RNA was added to plant cells, the on-target activity of engineered Cas9 increased. These stories show how close scientists are to making CRISPR-Cas9 both fast and specific, which will ultimately improve its safety. As highly effective versions of the system are developed, possibilities for engineered crops, cures for genetic disorders, and new, more powerful drugs start to feel within reach.