Research team evolves CRISPR-Cas9 nucleases with novel properties

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A team of Massachusetts General Hospital (MGH) researchers has found a way to expand the use and precision of the powerful gene-editing tools called CRISPR-Cas9 RNA-guided nucleases. In their report receiving advance online release in *Nature*, the investigators describe evolved versions of the DNA-cutting Cas9 enzyme that are able to recognize a different range of nucleic acid sequences than is possible with the naturally occurring form of Cas9 that has been used to date.

"In our paper we show that sites in human and zebrafish genes that could not previously be modified by wild-type Cas9 can now be targeted with the new variants we have engineered," says Benjamin Kleinstiver, PhD, a research fellow in the MGH Molecular Pathology Unit and lead author of the *Nature* paper. "This will allow researchers to target an expanded range of sites in a variety of genomes, which will be useful for applications requiring highly precise targeting of DNA sequences."

CRISPR-Cas9 nucleases consist of the Cas9 bacterial enzyme and a short, 20-nucleotide RNA molecule that matches the target DNA sequence. In addition to the RNA/DNA match, the Cas9 enzyme needs to recognize a specific nucleotide sequence called a protospacer adjacent motif (PAM) adjacent to the target DNA. The most commonly used form of Cas9, derived from the bacteria Streptococcus pyogenes and known as SpCas9, recognizes PAM sequences in which any nucleotide is followed by two guanine DNA bases. This limits the DNA sequences that can be targeted using SpCas9 only to those that include two sequential guanines.

To get around this limitation the MGH team set up an engineering system that allowed them to rapidly evolve the ability of SpCas9 to recognize different PAM sequences. From a collection of randomly mutated SpCas9 variants, they identified combinations of mutations that enabled SpCas9 to recognize new PAM sequences. These evolved variants essentially double the range of sites that can now be targeted for gene editing using SpCas9. Fortuitously, they also identified an SpCas9 variant that was less likely to induce the off-target gene mutations sometimes produced by CRISPR-Cas9 nucleases, a problem originally described in a 2013 study led by J. Keith Joung, MD, PhD, associate chief of Research in the MGH Department of Pathology and senior author of the current study. "This additional evolved variant with increased specificity should be immediately useful to all researchers who currently use wild-type SpCas9 and should reduce the frequencies of unwanted off-target mutations," Joung says.

"Perhaps more importantly," he adds, "our findings provide the first demonstration that the activities of SpCas9 can be altered by directed protein evolution. In fact, we show in our paper that the forms of Cas9 found in two other bacteria - Staphylococcus aureus and Streptococcus thermophilus - can also function in our bacterial evolution system, suggesting that we should be able to modify their functions as well. This work just scratches the surface of the range of PAMs that can be targeted by Cas9, and we believe that other useful properties of the enzyme may be modified by a similar approach, allowing potential customization of many important features." Joung is a professor of Pathology at Harvard Medical School.

More information: Engineered CRISPR-Cas9 nucleases with altered PAM specificities, *Nature*, DOI: 10.1038/nature14592

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