Team broadens utility of more compact CRISPR-Cas9 by increasing its targeting range

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A team of Massachusetts General Hospital (MGH) investigators has shown that a method they developed to improve the usefulness and precision of the most common form of the gene-editing tools CRISPR-Cas9 RNA-guided nucleases can be applied to Cas9 enzymes from other bacterial sources. In a paper receiving advance online publication in *Nature Biotechnology*, the team reports evolving a variant of SaCas9 - the Cas9 enzyme from the *Streptococcus aureus* bacteria - that recognizes a broader range of nucleotide sequences, allowing targeting of genomic sites previously inaccessible to CRISPR-Cas9 technology.

"The development of Cas9 variants with a broader targeting range is particularly important for applications requiring precise targeting of genomic sequences," says Benjamin Kleinstiver, PhD, a research fellow in the MGH Molecular Pathology Unit and lead and co-corresponding author of the *Nature Biotechnology* paper. "In addition, the coding sequence of SaCas9 is 23 percent smaller than that of SpCas9 - the version derived from *Streptococcus pyogenes* - a size difference that makes SaCas9 advantageous for potential therapeutic applications requiring delivery by viruses."

CRISPR-Cas9 nucleases are comprised of a short RNA molecule, 20 nucleotides of which match the target DNA sequence, and a Cas9 bacterial enzyme that cuts the DNA in the desired location. Along with the match between the RNA and DNA sequences, Cas9 needs to

recognize an adjacent nucleotide sequence called a protospacer adjacent motif (PAM). In a previous study reported earlier this year in Nature, the MGH team described a genetic system that enabled them to rapidly evolve SpCas9 to recognize different PAM sequences. While the naturally occurring SpCas9 recognizes a PAM sequence of the form NGG - in which N signifies any nucleotide and G is a guanine molecule - the MGH team was able to evolve versions of SpCas9 that recognize a broader range of PAM sequences, essentially doubling the range of targetable sites.

In their current study, the MGH team turned to SaCas9, which naturally requires the PAM sequence NNGRRT - in which R can be either adenine or guanine and T must be thymine - adjacent to its target DNA. Using an advanced form of the molecular evolution system described in the previous report, the MGH team succeeded in developing a variant they call KKH SaCas9 that recognizes PAM sequences with any nucleotide in the third position, increasing their targeting range two- to four-fold. The system was able to engineer these changes in PAM specificity without requiring advance knowledge of the precise structure of the SaCas9 enzyme, something that was unknown at the time this study was taking place.

"We now have shown that our directed evolution approach can be used to modify the PAM specificity of SaCas9, greatly expanding the number of genomic sites that can be accessed by this important Cas9 nuclease," says J. Keith Joung, MD, PhD, associate chief of Research in the MGH Department of Pathology and co-corresponding author of the *Nature Biotechnology* paper. "The ability to precisely target sites is important for researchers interested in disrupting small genetic elements or performing studies involving DNA repair by homologous recombination, an exchange of nucleotides between DNA strands, which best can be accomplished when the DNA break is close to the site of interest. We believe that our directed evolution approach provides an important

blueprint for altering the recognition properties of the wealth of Cas9 nucleases that exist in many bacteria." Joung is a professor of Pathology at Harvard Medical School

More information: Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition, *Nature Biotechnology*, DOI: 10.1038/nbt.3404

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