New CRISPR-Cas9 variants can respond to viral proteases

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Using a technique called circular permutation, researchers at the University of California Berkeley have created a new suite of Cas9 variants called Cas9-CPs, which will simplify design of Cas9-fusion proteins for diverse applications beyond simple DNA cutting, such as base editing and epigenetic modifications. The work appears January 10 in the journal *Cell*.

Through the same process, the researchers turned "always-on" Cas9 molecules into activatable switches that remain in the "off" position until activated by enzymes called proteases. The resulting protease-sensing Cas9s (ProCas9s) could reduce off-target effects and enable molecular sensing, as well as tissue- or organ-specific genome editing. The researchers demonstrated that ProCas9s can be used to detect viral proteases, potentially serving as a pathogen-sensing system capable of triggering an immune response.

"We're not stuck with what nature gave us with regards to genome-editing proteins," says senior author David Savage, a biochemist at the University of California, Berkeley. "These proteins can be elaborately optimized and turned into scaffolds possessing the right properties for use in human cells."

CRISPR-Cas proteins such as Cas9 are enzymes that protect bacteria from invading viruses. This bacterial defense system has been repurposed for genome-editing applications, such as inactivating genes, in a variety of cell types and organisms. Cas9 evolved to be a bacterial defense system, and it doesn't necessarily possess desired properties for genome editing in mammalian cells, such as extreme accuracy and precision in genome targeting, or the ability to control the spatial and

temporal activity of the enzyme.

While Cas9 fusion proteins have been constructed to efficiently edit nucleotides in DNA, or activate or repress transcription through epigenetic modifications—changes that affect gene activity without altering the DNA sequence—each new application requires an in-depth engineering approach with laborious optimization. Another major hurdle is that Cas9 is always in the "on" position. This lack of control over the protein's activity makes it difficult to target specific cells or tissues, can result in unintentional genome editing and greater off-target genome damage, and prevents the use of Cas9 as a molecular recorder of cellular events.

To overcome these obstacles, Savage and his team used circular permutation to reengineer the molecular sequence of Cas9, thereby achieving better control over its activity and creating a more optimal DNA-binding scaffold for fusion proteins. This Cas9-rewiring approach involves connecting the ends of the protein, i.e., its N- and C-termini, with a peptide linker, while concurrently splitting its sequence at a different position to create new, adjacent N- and C- termini.

The researchers found that Cas9 is highly malleable to circular permutation. Several regions of the protein possess hotspots that can be opened at numerous positions to generate a diversity of Cas9-CPs, which could serve as scaffolds for advanced fusion proteins. Currently, the N- and C- termini of Cas9 are fixed, and they are not ideally placed for fusion proteins to gain access to DNA. By contrast, the termini of the new protein scaffolds are placed closer to the DNA-interacting interface of the protein, and are optimized for the efficient construction of fusion proteins.

The researchers next generated ProCas9s by engineering the peptide linker, making the linker short enough to constrict the protein into an

inactive state, and then introducing sequences that could be cleaved by matching viral proteases. These ProCas9s were then tuned to serve as altruistic defense systems that could detect and respond to pathogens, generating massive DNA damage and killing infected cells when they were activated through cleavage of the linker peptide.

"I can envision various biomedical applications where the Cas9-CPs and ProCas9s will allow us to better understand disease processes and also enable safer translational applications of CRISPR-Cas genome editing and modification," says co-first author Christof Fellmann of the University of California, Berkeley.

In future studies, the researchers will work on developing Cas9-fusion proteins for base editing and epigenetic modifications. In addition, they plan to test whether ProCas9s can be used to build an entire synthetic immune system. It may also be possible to develop ProCas9s that are sensitive to endogenous proteases to target specific cells, for example, to edit the genomes of cancerous cells. "Our ProCas9 system is useful in any instance where having control of Cas9 would be useful," Savage says.

More information: *Cell*, Oakes and Fellmann et al.: "CRISPR-Cas9 Circular Permutants as Programmable Scaffolds for Genome Modification" www.cell.com/cell/fulltext/S0092-8674(18)31583-6 , DOI: 10.1016/j.cell.2018.11.052

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