GENOMICS

Putting the brakes on CRISPR–Cas9

Researchers identify biological inhibitors of Cas9 nuclease activity and block genome editing in cultured human cells.

Repurposing the bacterial antiviral defense system into a genome-editing tool has transformed biomedical research. The premise of the technique, known as CRISPR–Cas9, is simple; a Cas9 nuclease is guided by an RNA molecule (sgRNA) to a complementary genome sequence that is then cut and edited. Off-target effects currently hamper CRISPR–Cas9 applications. Though there are ways to limit these unwanted effects, approaches to stopping the activity of the Cas9 nuclease have just started to emerge.

In a recent collaborative study, a team led by Alan Davidson and Karen Maxwell from the University of Toronto and Erik Sontheimer from the University of Massachusetts identified Cas9 inhibitors from the bacterium *Neisseria meningitides*.

The team previously described small anti-CRISPR-associated (Aca) proteins that can block type-I CRISPR systems, which rely on Cas protein complexes not typically used as genome-editing tools. Searching for Aca homologs in *N. meningitides*, a bacterium that possesses a Cas9-dependent type-II-C CRISPR system, the researchers have identified three new anti-CRISPRs: $AcrIIC1_{Nme}$, $AcrIIC2_{Nme}$ and $AcrIIC3_{Nme}$. These proteins bind to NmeCas9 but not to Cas9 from *Streptococcus pyogenes* (SpyCas9), which belongs to a different Cas9 subtype. The interaction between any of these three anti-CRISPRs and NmeCas9 blocked DNA cleavage *in vitro*.

Forced expression of $AcrIIC3_{Nme}$ together with NmeCas9 and an sgRNA in HEK293 cells inhibited gene-editing activity. Using a fluorescently tagged nuclease-dead NmeCas9 mutant, the team further showed that $AcrIIC3_{Nme}$ prevented NmeCas9 or sgRNA genome binding and not DNA cleavage. However, given the great diversity in primary sequence between the three *N. meningitides* anti-CRISPRs, the researchers speculate that the other two inhibitors may act through different mechanisms.

Phylogenetic analysis of type-II-C Cas9 and anti-CRISPR homologs suggested that the majority of type-II-C CRISPR–Cas9 systems could be inhibited by at least one member of the three anti-CRISPR families. This encouraged the prediction that there might be at least one anti-CRISPR for any given Cas, including the SpyCas9 which is most commonly used for gene editing.

Potential applications of anti-CRISPRs are numerous and include temporal, spatial and inducible control of Cas activity, which would greatly limit unwanted gene editing.

Vesna Todorovic

RESEARCH PAPERS

Pawluk, A. *et al.* Naturally occurring off-switches for CRISPR–Cas9. *Cell* **167**, 1829–1838.e9 (2016).