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GENETIC ENGINEERING

A genome-editing off switch

> Cas9 activity could be limited to specific tissues or stages of development

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The CRISPR–Cas9 system has revolutionized molecular biology and biotechnology, rapidly becoming a commonplace laboratory technique. But concerns over off-target effects still limit its use. In a study published in *Cell*, Pawluk *et al.* identify natural regulators of Cas9 in *Neisseria meningitidis*, which could provide the key to controlling Cas9 activity.

Previous work identified small 'anti-CRISPR' proteins that are encoded by bacteriophages and can inhibit type I CRISPR–Cas defence systems of host bacteria, which function using Cas proteins other than Cas9. Now, analysing diverse bacterial species possessing type II CRISPR systems, which current CRISPR–Cas9 technologies are derived from, the authors conducted a search for homologues of the previously identified anti-CRISPR-associated (Aca) proteins Aca1 and Aca2.

In *N. meningitidis*, which yields a Cas9 with promising attributes for biotechnological applications, the authors found three potential anti-CRISPR genes in phage-derived mobile genetic elements, *AcrIIC1*$_{Nme}$, *AcrIIC2*$_{Nme}$ and *AcrIIC3*$_{Nme}$. The group conducted a series of *in vitro* assays demonstrating that the encoded anti-CRISPR proteins bind directly to the Cas9 protein in *N. meningitidis*. This interaction was specific for the type II-C CRISPR–Cas9 system, as the anti-CRISPR proteins failed to inhibit the commonly used type II-A CRISPR–Cas9 from *Streptococcus pyogenes*.

Having previously established that anti-CRISPR proteins can inhibit DNA cleavage, the researchers explored the possibility of using them as 'off switches' to control genome editing in cultured human cells. Working in HEK293T cells, expression of the anti-CRISPR genes successfully inhibited co-expressed *N. meningitidis* Cas9 from cleaving target DNA sequences.

Catalytically 'dead' Cas9 has been used to inhibit or activate gene expression without causing DNA damage. Using fluorescently tagged but catalytically inactive Cas9, the team observed that expression of the anti-CRISPR proteins prevented recruitment of Cas9 to the target locus.

Finally, Pawluk *et al.* investigated how the presence of inhibitors has helped to shape type II CRISPR–Cas system evolution. Phylogenetic analysis of Cas9 indicated that the majority of type II CRISPR–Cas systems could be susceptible to inhibition, and the authors propose that anti-CRISPRs may exist for all types of CRISPR–Cas systems.

The existence of inhibitors of type II CRISPR–Cas9 systems, as well as that of previously known inhibitors of type I systems, suggests that these inhibitors are widespread in nature and may exert their effects through different mechanisms. From an evolutionary perspective, the inhibitors and CRISPR–Cas9 may operate in an arms race: the bacteriophage-derived CRISPR inhibitors counteract Cas9 activity to enable successful infection of the host.

With regard to biological applications, the ability to control Cas9 activity, including selectively turning its activity off, opens up the possibility of much tighter regulation of the CRISPR–Cas9 system. For example, Cas9 activity could be limited to specific tissues or stages of development. Importantly, improved control of genome editing and gene expression manipulation would lessen the risk of off-target activity by Cas9.

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