CRISPR Meets Its Match

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Anti-CRISPR proteins could help control the gene-editing system.

CRISPR, the technology behind the 2020 Nobel Prize in Chemistry, confirmed what nearly a decade of research had already shown—that CRISPR has led to a revolution in the life sciences. The method offers a cheap, fast, and easy way to make edits in precise spots in genomes and could be game-changing for treating genetic disorders and cancer, and for other applications. However, CRISPR faces safety concerns because scientists have only limited ways to control the technique.

Now scientists have found that a dazzling variety of naturally occurring proteins, known as anti-CRISPRs, could offer ways to exercise that control. These proteins could potentially be delivered after gene therapy to stave off harmful side effects or help direct therapies to the right parts of the body. Anti-CRISPRs could also play a role in efforts to spread beneficial genes through a population—to control insects, for example. And they have several advantages over other CRISPR control methods; for example, they can be genetically encoded into a cell.

Clustered regularly interspaced short palindromic repeats (CRISPRs) are segments of bacterial DNA that are part of ancient microbial defense systems. Bacteria use RNA molecules derived from CRISPR segments, along with a number of CRISPR-associated (Cas) enzymes, to identify foreign DNA and slice it apart.

In 2012, scientists revealed a way to apply CRISPR RNAs and the Cas9 enzyme to cut the DNA in a cell’s genome in a precise spot. Essentially, all one needs is to introduce Cas9 and a guide RNA—which directs the enzyme where to cut—into cells.

But the longer a CRISPR system remains active in cells, the more likely it is to edit parts of a genome other than its intended target, yielding potentially dangerous side effects.

A cryo-electron microscopy reconstruction shows the anti-CRISPR protein AcrIIA4 (red) binding to a complex consisting of the Cas9 enzyme (white) and a guide RNA (orange). Adapted from Sci. Adv. 2017, DOI: 10.1126/sciadv.1701620 (CC BY-NC 4.0).

In addition, many of CRISPR’s potential therapeutic applications require edits in specific tissues; CRISPR activity in other tissues is at best useless and at worst hazardous.

"Think of any technologies humans use—TVs, computers, microwaves...they all have an on and off button for a reason,” says molecular microbiologist Rafael Pinilla Redondo of the University of Copenhagen. “You want to have a way to control these genome-editing technologies, or else the consequences would potentially be disastrous.”

“We believe anti-CRISPRs are very important for the development and success of CRISPR applications,” says synthetic
biologist Hirohide Saito of Kyoto University. “Anti-CRISPRs will be used in many fields but be especially useful in the field of biomedicine, where safety is required.”

From discovery to applications
Microbiologist Joseph Bondy-Denomy discovered anti-CRISPRs nearly a decade ago, when he was a doctoral student at the University of Toronto. He was investigating phages, viruses that infect bacteria. After the phages he was studying wove their DNA into bacteria—the kind of insertion that CRISPR usually prevents in bacteria—they made their hosts more resistant to other viruses, quelling competition. He unexpectedly discovered that the phages generate proteins that suppress the CRISPR-Cas system. Bondy-Denomy, now at the University of California, San Francisco, and colleagues later dubbed these molecules anti-CRISPRs.

To date, scientists have discovered more than 50 distinct families of anti-CRISPRs. All known anti-CRISPRs are small proteins, typically 50–150 amino acids in size. They bear little resemblance to one another in terms of sequence or structure, suggesting they all evolved independently. “The sheer breadth of inhibitory mechanisms that are in play with anti-CRISPRs is really remarkable,” says molecular biologist Erik J. Sontheimer of the University of Massachusetts Medical School. Some suppress the Cas enzyme’s ability to bind to DNA, whereas others prevent the system from cleaving DNA or interfere with the guide RNAs it relies on.

Anti-CRISPRs are drawing the most attention for regulating CRISPR therapies that aim to remove problem genes. “One very commonly invoked concern about CRISPR genome editing is accuracy,” Sontheimer says. “Cas9 can target the site you want, but the concern is that it may also end up mutating other sites you don’t want it to.” After delivering Cas9 and editing the desired site on the genome, a scientist can deliver an anti-CRISPR to shut down the enzyme and suppress accumulation of off-target edits, he says.

For example, when Bondy-Denomy and his colleagues used CRISPR in vitro to remove genes from human leukemia cells, they found that when they added an anti-CRISPR protein 6 h afterward, they could reduce CRISPR’s off-target effects. Bondy-Denomy cofounded a company, Acrigen Biosciences, focusing on therapeutic applications for anti-CRISPRs “to inactivate CRISPR in the body when it is no longer needed,” he says.

Anti-CRISPRs may also help limit gene editing to desired tissues within the body. In 2019, three independent teams in Germany, Japan, and the United States modified anti-CRISPR proteins to curtail their activity when they were exposed to molecules that exist only in certain tissues—for example, in liver and heart cells. “In this way, we can decide which cell’s genome to edit,” says Saito, who led the Japanese team.

The U.S. group also showed that its strategy could work in adult mice, not just in cells. “This was the first demonstration that an anti-CRISPR could function in a tissue of an adult mammal in vivo,” says Sontheimer, who led the team.

Anti-CRISPRs could also help scientists control gene drives, a technique that uses CRISPR and other gene-editing technologies to spread desired genes throughout a population. Although researchers hope gene drives can improve public health by, for instance, eradicating disease-laden mosquitoes and ticks, gene drives have also raised concerns about their potential unforeseen environmental impacts. Researchers at Kansas State University have demonstrated they could use anti-CRISPRs to halt a gene drive in yeast, decreasing its activity by more than 99.9%.

Anti-CRISPRs may even help address fears that CRISPR might get co-opted for use in biological weapons against people or the environment. “If we have a good arsenal of ways to stop and regulate CRISPR-Cas, they could serve as antidotes for these potential bioterrorism events,” Pinilla Redondo says.

Scientists have developed several ways to engineer anti-CRISPRs to exert even more control over CRISPR. For example, in 2018 molecular biologist Dominik Niøpek of
the Technical University of Darmstadt and his colleagues combined an anti-CRISPR with a light-sensitive molecule from oats as a way to switch the protein on and off using light. This optogenetic approach gives researchers “very precise spatial and temporal control of CRISPR gene editing from outside the body,” Niopke says. In addition, in 2019, Bondy-Denomy and his colleagues showed they could chemically control anti-CRISPRs by altering one so that it would destabilize in the absence of a small molecule known as Shield1, a commercial compound often used to break down specially tagged proteins.

One limitation of most anti-CRISPRs is that each molecule can suppress just one Cas protein at a time. But in 2019, a Chinese team and a U.S. group each discovered anti-CRISPR enzymes that could disable multiple copies of a Cas protein by slicing up their guide RNAs. “These enzyme anti-CRISPRs may potentially have a lot more use in biotechnology,” says biochemist Dipali Sashital of Iowa State University. “One could imagine using them to quickly shut down all Cas9 proteins, as opposed to using other anti-CRISPRs that would take longer or require much larger amounts.”

The future of anti-CRISPRs

Despite their potential, anti-CRISPRs’ usefulness in therapeutic applications remains uncertain. For instance, although two anti-CRISPRs have proven safe so far for use in mice, further research is needed to see if they might be toxic or trigger dangerous immune responses in humans, Niopke says.

Also, scientists are developing many other ways of controlling CRISPR, such as with small-molecule drugs, or by modifying Cas enzymes themselves to make them controllable via light or other means. The ultimate value of anti-CRISPRs could range “from not at all important to incredibly important—it’s hard to predict right now,” Bondy-Denomy says. “Control of CRISPR is needed—whether that’s through anti-CRISPRs or other methods, we’ll see at the end of the day.”

Still, anti-CRISPRs might stand out from these competitors in several ways. For example, Niopke says that “a big advantage of anti-CRISPRs is that they are fully genetically encodable, so they can be used as part of genetic circuits; chemical inhibitors cannot be used in this way. Furthermore, Bondy-Denomy notes that the genes for anti-CRISPRs can be encoded alongside and delivered with those for CRISPR-Cas systems, whereas other approaches will need a separate mode of delivery, potentially complicating their use.

Another advantage is that several anti-CRISPRs are each known to inhibit multiple variants of its target Cas enzyme. Such broad-spectrum activity could help scientists control multiple CRISPR-Cas systems—whether natural or engineered—without needing to reengineer each Cas protein for a desired application, Niopke says. Moreover, the variety of anti-CRISPR mechanisms means that researchers can select and optimize anti-CRISPRs to their needs, he adds.

“Small-molecule inhibitors can be exceptionally effective because of the ease and speed with which you can apply them,” Sashital says. “But anti-CRISPRs are most likely going to be quite specific for their particular Cas proteins, whereas small-molecule inhibitors might always have potential off-target effects. Certainly, pursuing both, in terms of developing CRISPR inhibitors, is the way to go.”

Although it remains an open question as to whether anti-CRISPRs might have direct clinical applications, these proteins might also indirectly help scientists develop therapies.

For example, Sontheimer and his colleagues wanted to develop a viral delivery mechanism that would use CRISPR-Cas9 to both cure genetic disorders and destroy the viral vector itself so that the entire package does not survive long enough to have off-target effects. But the scientists manufacture these viruses by incubating their genetic material in bacteria, and sometimes the viral DNA can inadvertently produce Cas9, demolishing the viruses before the researchers can use them in experiments. It is like trying to bottle jars of nitroglycerin that might easily annihilate themselves.

So the researchers included an anti-CRISPR that prevented the Cas9 from recognizing target viral DNA sequences, protecting the viruses during incubation. In a preprint, they showed that their viruses could treat two genetic disorders in mice and then self-destruct over time, reducing the risk of long-term side effects.

“Anti-CRISPRs are already proving very, very useful for research purposes,” Sontheimer says.

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