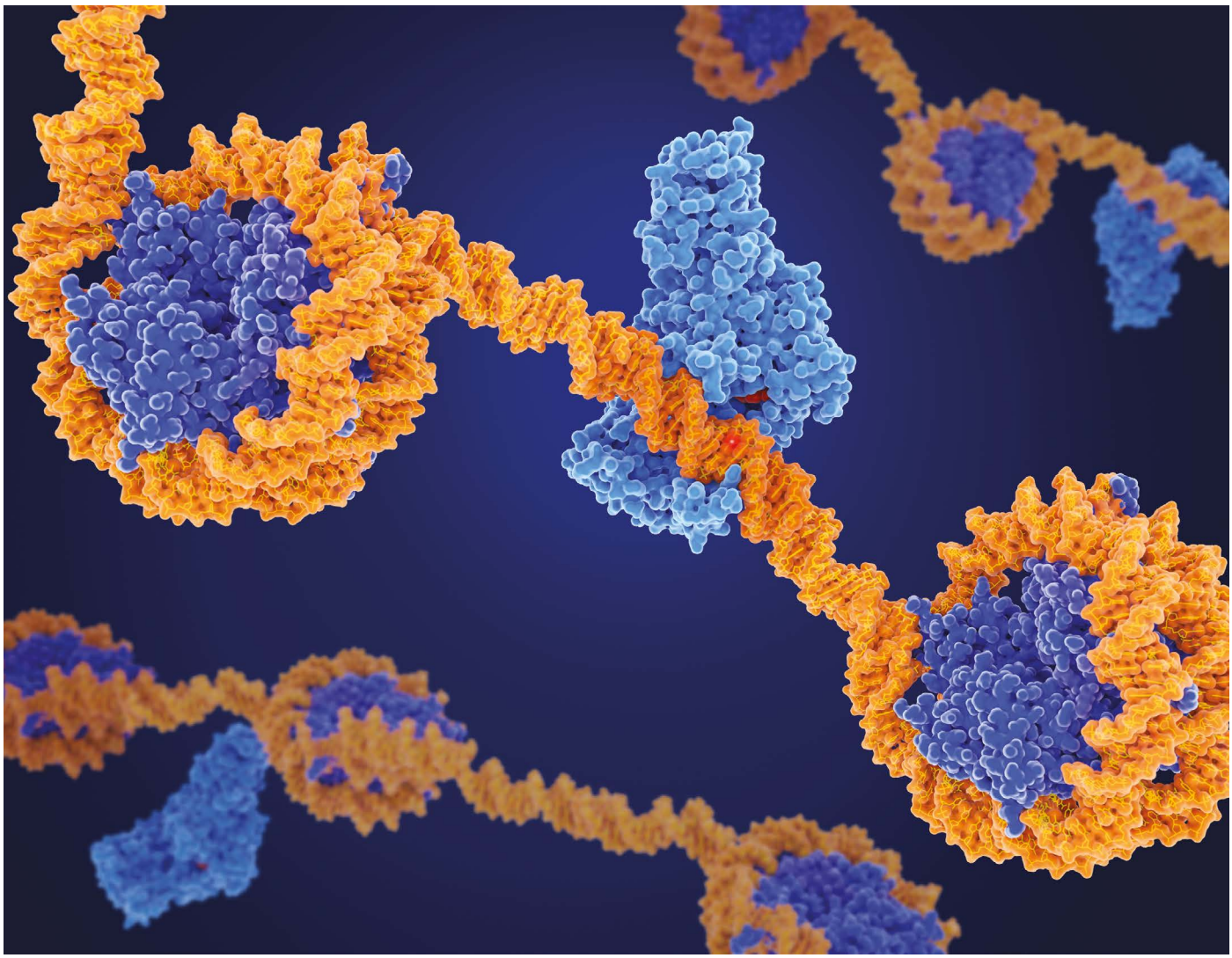

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Epigenetic modifications to DNA (yellow), such as those catalysed by DNA methyltransferase enzymes (blue, centre) control gene expression.

# EPIGENETIC EDITING MAKES ITS MARK

Researchers are rewriting the chemical tags on DNA and chromatin to tune gene expression. **By Amber Dance**

Marianne Rots first started talking about epigenome editing on the conference circuit about two decades ago. If natural epigenetic marks such as DNA methylation and histone acetylation control gene expression, she reasoned, then artificially modifying those marks should allow scientists to adjust that expression. But many of her peers scoffed, assuming that epigenetic tags could not trigger such changes. "We really had to fight dogmas, that people thought, this will never, ever work," says Rots, an epigeneticist at the University Medical Center Groningen in the Netherlands.

Then came the CRISPR–Cas system. Although the technology first made a splash in gene editing, scientists such as Rots now use it to precisely target epigenetic editors to any DNA sequence. Suddenly, epigenetics researchers could not only silence or activate genes, but also dial gene expression up or down. More than a dozen companies are now exploring epigenetic-editing technology, and a handful of early-stage clinical trials are under way.

Tunable and flexible, epigenetic editing stands in elegant contrast to gene editing. CRISPR's Cas enzyme slashes genetic material apart, then relies on cellular systems to repair it, hopefully incorporating the desired edit. Epigenetic editing is gentler, leaving the DNA strands and code unchanged, and can induce either temporary or permanent changes.

"I find epigenome editing to be much more sophisticated, much more complex," says Charles Gersbach, a biomedical engineer at Duke University in Durham, North Carolina. "There are just so many more things that you can do with epigenome editing that aren't necessarily doable with genome editing."

For example, in epigenetic editing, adding a handful of guide RNAs — the nucleic acids that target the Cas enzyme to a specific genomic location — to the machinery allows scientists to alter several sites at once. By contrast, because gene editing requires creating a double-stranded DNA break, editing several gene sequences at once risks the pieces reconnecting in unnatural, dangerous configurations.

Researchers are using epigenetic editing to explore the intricacies of gene expression and to develop therapies. Plant scientists are fiddling with epigenomes, too, to create crop variants that differ not in DNA sequence, but in gene activity. But the epigenome is complex, and many projects require trial and error.

"We really don't understand the rules," says Rots. "We cannot predict the final outcome of our biological experiments."

## From the bench...

It's no wonder the rules are unclear: "The epigenetic modifications are numerous," says Elizabeth Heller, a neuroscientist at the University of Pennsylvania Perelman School of Medicine in Philadelphia. The DNA molecule can be modified by methylation — which can silence genes — and its associated histones are subject to dozens of possible chemical decorations, including acetylation, phosphorylation and biotinylation, each of which has its own mode of action. "Even at a given gene, there's usually many of them, changing all at once," says Heller. In human cells, gene expression is managed by about 900 chromatin regulators and 1,600 transcription factors, proteins that bind to DNA sequences to control the rate of gene expression.

Therefore, one of the first applications for epigenetic editing is in probing the mechanics of epigenetics itself. Heller, for instance, studies the genetic impacts of cocaine use. Preliminary results suggest that when mice are exposed to the drug and later denied it, histone methylation is diminished at a particular genetic locus, and production of the corresponding mRNA transcript increases[1]. This suggests that the withdrawal of cocaine caused the epigenetic changes, which then altered gene expression. But to dissect precisely what's happening, she needs to modify epigenetic marks, one at a time or in concert. Epigenetic editors are just the ticket.

The basic editor has two key parts. The first is a targeting mechanism — generally a version of CRISPR's Cas enzyme that cannot cut DNA, along with a guide RNA to bring it to the locus.

The other is the effector, which makes the edit or otherwise influences gene expression. Some effectors are enzymes that directly modify DNA and histones, such as methyltransferases and demethylases. Other effectors don't act directly on the DNA itself, but recruit or block transcriptional machinery — artificial transcription factors (ATFs), as Heller and Rots call them[2].

For temporary changes, Heller says, researchers can introduce plasmids that encode the editor or ATF into cells. The plasmids can be delivered in a few ways, for example as DNA or packaged inside short-lived viral vectors such as herpes simplex virus. The expression of the editor or ATF, and any resulting changes, will last for a week or so.

For longer-term changes, researchers might use lentiviral vectors, which integrate their genes into the host-cell genome. Or they can create transgenic cell lines or organisms with genomes that have been modified to express the editor or ATF.

"Having all of those options does make it a little daunting," says Gersbach. "But also, it's really powerful, in creating a lot of flexibility."

Rots recommends starting with a couple of commonly used effectors, many of which are available from the reagent-distribution service Addgene, based in Watertown, Massachusetts. To turn gene expression up, consider VP64 and its related constructs, activators based on a transcription factor from herpes simplex virus 1. To decrease expression, try CRISPRoff, built from the transcriptional repressor KRAB domain and a DNA methyltransferase complex, which catalyses methylation.

**"Having all of those options does make it a little daunting. But also, it's really powerful in creating a lot of flexibility."**

Gersbach used KRAB, VP64 and another gene activator called TET1 in a 2025 study to probe gene regulation in Prader–Willi syndrome, which causes ongoing hunger, among other symptoms. The disease occurs when there's a deletion in the copy of chromosome 15 inherited from the father. All the genes are present on the maternal chromosome 15, but they're methylated, or turned off. Rather than trying to reactivate each gene across 5–6 megabases of genetic material, Gersbach is seeking a "master switch" to control them all — a strategy he hopes could one day lead to a therapeutic.

To investigate how the region is regulated, the researchers paired more than 11,000 guide RNAs with either VP64 to activate expression of the silenced maternal allele or KRAB to repress the normal paternal version. Hits from the screen and further work with the TET1 activator identified a single genomic location at which targeted demethylation activated silenced genes, even after the editor had dissipated[3].

## To the clinic...

At the San Raffaele Telethon Institute for Gene Therapy in Milan, Italy, molecular biologist Angelo Lombardo also has therapeutic ambitions for epigenetic editing. In 2016, Lombardo described a method that he calls "hit and run" editing to achieve long-term changes from transiently expressed effectors[4]. Like CRISPRoff, the system relies on KRAB and a methyltransferase enzyme, as well as customizable DNA-targeting molecules. Short-term expression of these constructs in cultured cells placed repressive marks on the DNA and histones of target genes, shutting them off for good. The effects last for months, across several rounds of cell division, Lombardo says.

Lombardo's team used the same system to deactivate the *Pcsk9* gene in mice[5]. Expressed in liver cells, *Pcsk9* regulates cholesterol levels; small-molecule PCSK9 inhibitors can treat high cholesterol, and gene-editing strategies to disable the gene are in trials. Lombardo's group used lipid nanoparticles to deliver messenger RNA encoding their epigenetic editor to mouse livers, which almost halved PCSK9 levels. A single treatment created effects lasting nearly a year. The researchers removed part of the liver in four mice, forcing the organ to regenerate — and the new tissue maintained the epigenetic repression, showing that it can be passed from mother cell to daughter.

Lombardo co-founded nChroma Bio in Boston, Massachusetts, to further develop epigenetic treatments such as this one, and the company is taking the PCSK9 programme through its next steps. In mice, nChroma researchers silenced expression of a human *PCSK9* transgene, leading to a reduction of more than 98% in protein levels for at least a year — and they could reactivate the gene, too, by removing the silencing methyl marks[6]. In the same study, cynomolgus monkeys were given the treatment, which slashed PCSK9 levels by about 90%, and levels of low-density lipoprotein, or 'bad', cholesterol dropped by about 70%.

Both nChroma and Tune Therapeutics, which was co-founded by Gersbach and is based in Seattle, Washington, are trialling epigenomic treatments for hepatitis B. Infected liver cells contain many copies of the viral genome: some are integrated into the host chromosome, and others exist outside it. The idea behind these treatments, Gersbach explains, is to silence the viral genomes and prevent production of viral particles, thus leading to a "functional cure". At the European Association for the Study of the Liver conference in Barcelona, Spain, in May, Tune reported that, in an ongoing, early-stage trial, the higher doses of the treatment eliminated several viral protein and RNA biomarkers for up to 17 months.

Back in the laboratory, Lombardo is now combining epigenetic editing with gene editing in what he calls an all-in-one "crazy platform" for cancer therapeutics. In CAR-T therapy, immune cells are reprogrammed with chimeric antigen receptors (CARs) that target cancer cells. But it's also helpful to inactivate

other genes that could inhibit the CAR T cells' immune actions or cause them to attack the wrong targets. Lombardo reasoned that only the addition of the CAR requires actual genetic editing; silencing other genes can be accomplished through the gentler epigenetic approach.

The trick was to design a system that could cleave a single target site to insert the CAR gene while targeting epigenetic editors to other genomic locations. Lombardo's solution was to use guide RNAs of varying lengths and an active Cas9 enzyme that could slice DNA. He used a full-length guide RNA to target the desired CAR locus, which Cas9 cleaved during full-on genetic editing. Truncated guide RNAs targeting other sites were sufficient to recruit the epigenetic effectors — again, KRAB and methyltransferases — without cutting DNA. In May, the scientists reported that they had successfully expressed CARs and epigenetically silenced two other genes in 80% of cells in culture[7].

## To the greenhouse

Epigenome editing is also making inroads in the agricultural sciences. Many plant traits, such as height and yield, are controlled by epigenetics, explains Jian-Kang Zhu, a molecular geneticist and president of Macau University of Science and Technology in China. Editing plant epigenomes could create valuable breeds without having to alter the DNA sequence.

The goals and concerns in plant epigenetic editing are different from those in animal editing, says Zhaobo Lang, an epigeneticist at the Southern University of Science and Technology in Shenzhen, China. She and Zhu want changes to be not just long-lasting, but heritable, so that they affect future generations. Thus, they aim to edit the plants' germline. Another difference is that they don't necessarily care whether the editors have off-target effects: if a change is detrimental, they can simply trash the affected plants. But, sometimes, those unintentional changes are beneficial, says Zhu.

Lang, Zhu and their colleagues have spent more than a decade building tools, mixing and matching diverse DNA-targeting modules, effector proteins and co-factors that enhance effector action in plants. They tested these in the model plant *Arabidopsis thaliana*, silencing and activating reporter genes that altered flowering timing. The work took so long, Zhu says, in part because they ensured that the changes lasted for at least three generations, and sometimes ten or more. (A single *A. thaliana* generation takes four to five weeks.)

The resulting toolbox, published in December in *Nature Communications* and available at Addgene, includes five enzymes that add methyl groups to DNA and six that remove them, with a range of specificities and efficiencies[8].

Researchers will probably have to modify the tools to be compatible with other species that they're working with, Lang and Zhu advise. For one thing, it's easy to transfer genes into *A. thaliana* by dunking the flowers in a suspension of *Agrobacterium*, a bacterial genus that delivers transgenes to germ cells. But that won't work for many crops. The gene promoter that drives editor expression is *Arabidopsis*-specific, too.

Epigenetics researcher Elizabeth Heller.

DANIEL BURKE

## Practical considerations

Although epigenetic manipulation once seemed impossible, the field is now moving fast — perhaps too fast, in the case of clinical trials, Rots says. She worries that failures in human studies set the whole field back. "We still have so much to learn and to understand," she says.

For now, that means that finding or designing the right tool could require being open to different options. Lombardo's team spent two years attempting to silence *Pcsk9* using CRISPR–Cas-based targeting before trying a zinc-finger nuclease instead. Zinc fingers are an older technology and are not as simple to design, but the constructs are smaller, making them easier to deliver to tissues. "The zinc finger worked, the first test we did," he recalls.

> **"In terms of activity, we're very happy. Epigenetic editors, they really work, and they really work well."**

The biggest challenge, scientists say, is delivering the genes for the effectors to target cells or tissues. "It takes a year of a PhD to get the right delivery protocols, and that is very frustrating," laments Rots. To ascertain whether the cargo even made it in, scientists can check for the presence of editor components, such as Cas9, in target cells.

The next question is: did the effector find its target and avoid off-target sequences? Chromatin immunoprecipitation with DNA sequencing can help here, says Pilar Blancafort, a cancer biologist at the University of Western Australia in Crawley. Bioinformatics tools such as dsNickFury2 and Elevation can identify potential off-target sites for given guide RNAs, helping scientists to determine whether unexpected epigenetic changes are indeed the result of off-target actions.

To control for editor activity, apply a deactivated version of the editor enzyme, suggests Heller. And bisulfite sequencing can uncover changes to methylation patterns, adds Lang. Then, quantitative reverse-transcription polymerase chain reaction or RNA sequencing can reveal any impact on RNA levels.

Finally, to ensure that any altered phenotypes are a result of the edit, Gersbach suggests trying alternative tools that should have the same effect, or a different guide RNA targeting the same genomic region, and seeing whether the results match.

All that said, epigenetic editing can work beautifully, says Blancafort; she finds the process quick and efficient. Because it's the epigenome that ultimately determines cell type, she's using editing to nudge breast cancer cells from a dangerous mesenchymal state to an epithelial state that is more receptive to treatment[9]. As part of that ongoing project, she says, epigenetically silencing the genes encoding four transcription factors altered the epigenetic tags at 100,000 cytosine–guanine sites in the genome. "In terms of activity, we're very happy," Blancafort says. "Epigenetic editors, they really work, and they really work well."

To bring that joy to more researchers, Rots and others are developing guidelines that will lay out which genes will respond to which effector domains. She's participating in one such project, the Dutch Epi-Guide-Edit consortium, which is working to define editing rules that the group describes as "precise, predictable, and long-term". But it's challenging, she says: although the group aims to complete its project by June 2028, it has examined the impacts of individual effectors on only about 100 genes so far.

The complexity is no reason to be intimidated, says Gersbach: "Just jump in and get your hands dirty."

**Amber Dance** is a freelance science writer and editor in Los Angeles, California.

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**Correction**
This Technology feature gave the wrong affiliation for Angelo Lombardo.

Corrected 22 July 2026