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EPIGENETICS

# The genome unwrapped

*Epigeneticists are harnessing genome-editing technologies to tackle a central question hanging over the community — does their field matter?*

**BY HEIDI LEDFORD**

On 18 February, a consortium of more than 90 laboratories published a landmark catalogue of the chemical changes to DNA that are thought to influence whether and how genes are expressed. Called the Roadmap Epigenomics Project and sponsored by the US National Institutes of Health, the compendium offered an unprecedented look at the layers of coding that exist on top of the genetic code — collectively known as the 'epigenome' — in 127 different human tissues and cell types. The US$154-million project was viewed as a crucial step towards determining how this chemical code contributes to human health and disease. As researchers get to grips with the catalogue's contents, the project is also likely to provide a leap forward in pinning down one of the central mysteries of biology: how do cells with the same genetic instructions take on wildly different identities?

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It is still unclear what that epigenetic code actually does, and how it is generated. "I don't think it can be overstated how little we understand about how the epigenome works," says Charles Gersbach, a biomedical engineer at Duke University in Durham, North Carolina. "There are all of these epigenetic marks and we don't know what they are doing. Are they even necessary?"

After years of wondering, biologists such as Gersbach are now in a position to find out. By harnessing genome-editing technologies, they are able to interrogate the epigenetic control of gene expression with remarkable power and specificity. Researchers can make or delete epigenetic marks at will, and home in on RNAs and proteins that could play a hitherto unrecognized part in directing gene expression. And with these new capabilities, they hope to build an answer to a key question that has plagued the field of epigenetics since its inception — do epigenetic marks alter gene expression or do changes in gene expression alter the marks? "It's an absolutely legitimate question and we need to address it," says Luca Magnani, a cancer researcher at Imperial College London. "The answer is either going to kill the field, or make it very important."

## A THICKET OF COMPLEXITY

Epigenetics is not for the faint of heart. Where the genetic code offers simplicity and stability, with its four bases of DNA, passed down stably from one generation to the next, the epigenetic code is gnarly and dynamic. Dozens of different chemical modifications

decorate both the DNA and the histones — proteins that package the DNA into chromosomes. All of these marks can vary from cell to cell, influenced by age, developmental stage and the environment. "One of the biggest challenges in this area is knowing whether what you've observed is generalizable or if it's specific to that gene or that cell type or the culture conditions or the day of the week or the cycle of the moon," says Gersbach.

Getting to grips with such complexity could generate huge payoffs, and not just for basic research. For example, when scientists alter epigenetic networks, they can coax stem cells to take on a new identity — and perhaps, in the future, this could be used to treat disease. Similarly, genomic studies frequently point to the important role that the full collection of epigenetic patterns in a cell nucleus has in complex diseases such as diabetes or schizophrenia, notes Tim Reddy, a genomics researcher also at Duke University. "In a lot of these cases, it really seems to be not a DNA mutation that impacts the protein sequence, but a change in how genes are regulated," he says. Targeting these regulatory elements and altering their activity could one day yield a new approach for treating complex diseases, he adds.

Drugs that are thought to work by modifying the epigenome are already on the market in places such as the United States and Europe. Some inhibit enzymes that either add or remove acetyl groups from histones, and can treat a range of conditions from epilepsy to cancer. Whereas other drugs treat cancer by blocking enzymes that remove methyl groups from DNA.

But from a scientific standpoint, the problem is that no one knows exactly which epigenetic alterations lie behind these drugs' effectiveness. The drugs act globally over the entire genome, rather than being directed to any specific location, which makes it impossible to use them to determine the function of individual, or even regional, epigenetic changes. Some researchers even view the tolerability of the side effects of these liberally acting drugs (including those that inhibit enzymes called histone deacetylases, or HDACs) as suggesting that some epigenetic marks are not important in regulating gene expression. "If you can just eat an HDAC inhibitor, then exactly how important is that enzyme?" says Gersbach. "It's clear that we don't understand it very well."

## A CRISP, NEW DAWN

The enzyme components of genome-editing technologies offer a way forward because they allow researchers to focus on a single region of DNA. Before the editing system CRISPR–Cas9 became widely used, researchers targeted the epigenome by altering the FokI enzyme — the enzyme involved in the editing technologies zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). The first step was to disable FokI's capacity to cut DNA, without removing ZFNs and TALENs ability to home in on a target sequence. The incapacitated enzyme was then attached to another enzyme that could make or remove epigenetic marks. The outcome was an epigenetic enzyme targeted to a specific location in the genome — or, put another way, a chance to interrogate the function of specific epigenetic changes.

But ZFNs and TALENs can be difficult to work with, and results from experiments that use them have been slow to trickle in. ZFNs are also prone to creating unwanted, off-target alterations to the epigenome, notes Tomasz Jurkowski, a biochemist and epigeneticist at the University of Stuttgart in Germany. "You could not reach a final conclusion — maybe what you were seeing were secondary effects from somewhere else," he says.

Earlier this year, researchers reported that CRISPR–Cas9 could be adapted to do the same thing, but with less effort and uncertainty (N. A. Kearns *et al. Nature Meth.* **12,** 401–403; 2015). René Maehr, an immunologist at the University of Massachusetts Medical School in Worcester and his colleagues fused an enzyme called histone demethylase, which removes methyl groups from histones, to a deactivated Cas9 enzyme, and then programmed it to target regions of DNA believed to enhance the expression of certain genes. The result was a functional map of genetic 'enhancer' sequences that allows researchers to determine what these enhancers do, how strongly, and — most importantly — where they are located in the genome.

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Meanwhile, Gersbach, Reddy and their colleagues coupled an inactive Cas9 to an enzyme called an acetyltransferase, which attaches acetyl groups to histones — a process that is thought to turn genes on (I. B. Hilton *et al. Nature Biotechnol.* **33,** 510–517; 2015). Reddy says that he was surprised at the extent to which the expression of a target gene increased when a histone in an enhancer region was acetylated, given the uncertainty as to whether DNA marks are a cause or a consequence of such activation. "That result started to convince me that the acetylation of histones may be a direct cause of gene activation," he says.

But it will take many such studies before the community knows whether that result applies to other epigenetic marks. It is possible that some marks cause changes to gene expression, whereas others could merely be an effect of a change to gene expression. "It's hard to put it all into some neat package," says Steven Henikoff, a geneticist at the Fred Hutchinson Cancer Center in Seattle, Washington. "For all we know, they might have very minor effects on gene expression except in a few special cases."

## THE WAY AHEAD

Now, however, researchers have a tool to pick apart the detail. Because of its simplicity and versatility, CRISPR–Cas9 opens up an opportunity to launch the kind of large-scale projects needed to reach that level of understanding. "If we want to target a region in the genome, we can have that targeting molecule here tomorrow for five dollars," says Reddy. "We're going to get to march through every single one of these modifications and figure out what they actually do."

There will still be technical hurdles to overcome, cautions Gersbach. For example, the enzymes needed to make or erase epigenetic marks sometimes lose their activity when they are tacked on to inactive FokI. And, as epigeneticist Marianne Rots of the University of Groningen in the Netherlands notes, Cas9 is relatively large as proteins go. As a result, it can have trouble accessing stretches of DNA that are especially tightly wound.

Despite this, there is still plenty of room for ambitious projects. Jeremy Day, a neuroscientist at the University of Alabama in Birmingham, is using CRISPR–Cas9 to study the long-lasting epigenetic changes associated with addiction that occur in the brain. His aim is to use recently described systems in which light activates CRISPR–Cas9. This would allow him to control where and when an enzyme adds or removes any given epigenetic mark. For Day, this advance means that the marks of addiction on the brain could one day be reversed, without hindering the ability of a patient to feel pleasure in response to other stimuli. "You don't want to just deaden people," he says. "With these very specific tools we can find out the critical modifications that perpetuate addiction." And, more broadly, for the field of epigenetics, this light activation technology offers a kind of revolutionary power. "It will allow us to learn a lot about the basic biology of those epigenetic marks: how long do they last? How much of that modification do you need to affect the gene?" Day adds.

Although any therapeutic application of CRISPR–Cas9 to epigenetics is still in the distant future, the rapid pace of the field is already defying expectations. Jurkowski, for one, started his lab in 2012 just before the first papers showing CRISPR–Cas9 genome editing in human cells were published. Like many researchers, Jurkowski then took up CRISPR research in 2014, but has been scooped twice by competing labs in less than two years. He takes the competition in stride — it is the price of entry into the fast lane. Epigenetics is on the verge of a revolution, he says. "This is just the beginning," he says. With just a little more time, "It will develop into a completely new field." ■

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