Studying epigenetic regulation at the single-molecule level

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Scientific illustration of a chromatin close-up. A portion of the chromatin is shown as open, allowing for transcription factors to bind to DNA (lilac spheres). Depending on whether the DNA is methylated or not (white spheres), transcription factors have different sensitivities that affect their function. Credit: Joana Gomes Campos de Carvalho/EMBL

If one imagines the genome as an instruction manual for the functioning

of a cell, every page of this manual is covered with annotations, highlights, and bookmarks. The role of some of these marks remains mysterious—do they actively direct the reader to the right place at the right time, or do they merely indicate the pages the reader has already visited?

This subtle distinction in the language of the cell can play an important role in its survival and function. As researchers from the Krebs group at EMBL Heidelberg have now shown, one such annotation—DNA methylation—exerts a highly selective layer of control on the expression of genes, one that varies according to cell type and fate.

In the analogy above, the annotations, highlights, and bookmarks represent what scientists call 'epigenetic marks', whereas the 'reader' is usually the complex molecular machinery responsible for gene expression. The latter includes specialized proteins known as transcription factors.

When a particular region of DNA needs to be expressed, the area surrounding it undergoes physical and chemical changes, making it more accessible to such molecular machines. While DNA methylation is found across the genome, whether and how it affects this accessibility at specific genomic regions remains relatively unexplored.

"Our group is interested in the fundamental mechanisms that regulate gene expression," said Arnaud Krebs, Group Leader at EMBL Heidelberg. "We are particularly interested in cis-regulatory elements like enhancers—DNA regions that control the activity of genes."

Krebs' team was intrigued by the fact that while DNA methylation is often reduced at active enhancers, the cause-effect relationship between the two remains unclear. Does the activation of these DNA regions lead to a removal of methylation? Or does the reduction in methylation itself

drive the activation?

To investigate this, the team used a high-resolution technique developed in their lab—single-molecule footprinting. This method allowed them to simultaneously measure DNA methylation, accessibility, and transcription factor binding, at the level of single DNA molecules. They applied this across the whole genome in multiple cell types, including mouse embryonic stem cells and differentiated cells. This combination of scale and resolution allowed the scientists to gain a deeper understanding of DNA methylation's role in gene regulation in a living cell.

The team found that while the accessibility of ~97% of the enhancers they studied was insensitive to DNA methylation, about 3% required the absence of DNA methylation to get activated. At these sites, methylation reduced DNA accessibility and directly prevented the binding of transcription factors. The identity of these methylation-sensitive enhancers varied across cell types and stages.

"The 3% of enhancers that seem to be regulated by DNA methylation are enriched for cell-type specific enhancers. We think they are connected to genes that are important for cellular identity," said Elisa Kreibich, Ph.D. student in the Krebs group and first author of the study, now published in *Molecular Cell*.

"By making our measurements at the level of single molecules, we can figure out the connections and interactions between the layers of gene regulation that exist in a cell," added Krebs. "While DNA methylation has often been used as a marker for cellular processes, including those involved in cancer, our study shows where it is truly instructive, rather than simply indicative."

More information: Elisa Kreibich et al, Single-molecule footprinting

identifies context-dependent regulation of enhancers by DNA methylation, *Molecular Cell* (2023). DOI: 10.1016/j.molcel.2023.01.017