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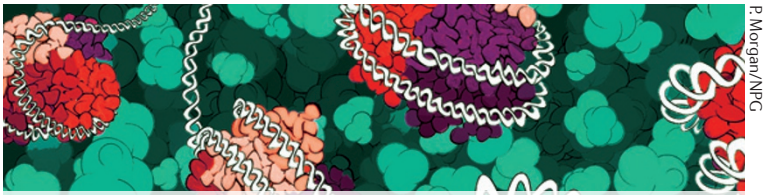

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GENE REGULATION

Chromatin editing reveals enhancer targets

> This study provides a useful new tool to identify enhancer target genes in their endogenous context

A crucial question plaguing enhancer research is how to identify the target genes of the many putative enhancers that have been discovered in genome-wide studies. A new study attempts to address this issue by creating a new tool that specifically modifies the chromatin signatures of particular enhancers to identify their target genes.

Mendenhall and colleagues investigated active enhancers, which are characterized by histone H3 lysine 4 monomethylation (H3K4me1) and dimethylation (H3K4me2), and H3K27 acetylation (H3K27ac). They reasoned that if they could modify these epigenetic marks, which are thought to be involved in enhancer function, then they could identify the genes that are targeted by these enhancers through the resultant changes in their expression.

To do this, the authors combined the chromatin-modifying properties of lysine-specific demethylase 1 (LSD1), which can demethylate H3K4, with the DNA-binding properties of transcription activator-like effector (TALE) repeat domains, which can be engineered to bind to specific regions of DNA. Specifically, they created fusion proteins of TALE repeat domains and LSD1 to produce chromatin-modifying tools that were unique for each enhancer examined. These constructs could then be transiently transfected into human cell lines to study enhancers in their endogenous contexts.

TALE–LSD1 fusions were validated as chromatin-editing tools using an enhancer at the stem cell leukaemia (*SCL*) locus, which is marked by high levels of H3K4me1, H3K4me2 and H3K27ac. The authors confirmed the binding specificity of a TALE–LSD1 fusion protein that was targeted to this enhancer. Intriguingly, when they examined the effects of this fusion protein on chromatin marks, LSD1 was found not only to have demethylase activity but also to reduce levels of H3K27ac. This finding expands the use of TALE–LSD1 fusion proteins as chromatin-editing tools. Finally, the modification of histone marks by the fusion protein was shown to affect the expression of the target gene of this enhancer.

The authors then extended their study to alter the chromatin at 40 putative enhancers in order to identify their target genes. Using chromatin immunoprecipitation followed by quantitative PCR they found that, in the presence of TALE–LSD1 fusion proteins, 65% of these putative enhancers had reduced levels of H3K4me1, H3K4me2 or H3K27ac at their specific target loci. The authors then looked in more detail at nine of these TALE–LSD1 fusion proteins that showed the most dramatic reduction in these chromatin modifications. Using RNA sequencing they showed that four of the fusion proteins caused a neighbouring gene to be downregulated by at least 1.5-fold, thus indicating that these genes are targets of the enhancers. The authors were unable to determine whether the remaining five fusion proteins had weak effects below their detection threshold or whether these enhancers did not regulate any genes in this particular cell type. Addressing these issues will be important to further refine this method.

This study provides a useful new tool to identify enhancer target genes in their endogenous context, as well as to gain insights into chromatin biology in general.

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ORIGINAL RESEARCH PAPER Mendenhall, E. M. *et al.* Locus-specific editing of histone modifications at endogenous enhancers. *Nature Biotech.* http://dx.doi.org/10.1038/nbt.2701 (2013)