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TECHNIQUE

CRISPR CAPTURE for multi-omic probing of genomic loci

> a promising multilayered approach for understanding gene regulation mechanisms

It is now widely appreciated that alterations to gene regulation can be important for evolution, development and pathological transitions. However, a comprehensive understanding of these phenomena will require detailed molecular characterizations of how regulatory elements control their target genes. A new study adapts the CRISPR system for a multi-omic dissection of regulatory elements and their interactions with target loci.

Liu *et al.* sought to direct the CRISPR-associated protein Cas9 to regulatory elements as an affinity tag to enable subsequent molecular characterization of the surrounding chromatin. Onto a nuclease-dead Cas9 (dCas9), they engineered a biotin acceptor site so that locus-bound dCas9 could be biotinylated *in vivo* by the biotin ligase BirA and isolated by streptavidin-based capture, followed by analysis of the locus-associated proteins and nucleic acids by various methodologies. They termed their approach CRISPR affinity purification *in situ* of regulatory elements (CAPTURE). Key features are that the high-affinity biotin–streptavidin interaction provides higher sensitivity and specificity than antibody-based strategies to isolate locus-bound proteins, and that guide RNA (gRNA)-based targeting enables multiple regulatory loci to be targeted simultaneously.

The team primarily focused on using CAPTURE to dissect regulation at the human β-globin locus, which consists of five β-like globin genes that are controlled by a shared cluster of five enhancers. The authors co-expressed dCas9, gRNAs and BirA in human K562 cells, and optimized proteomic analysis following CAPTURE of individual enhancers or promoters. They found known erythroid transcription factors and chromatin remodellers at β-globin enhancers, but also identified novel regulators such as nucleoporin nuclear pore components. Functional relevance was confirmed by showing that knockdown of these components altered β-globin gene expression. Furthermore, the authors classified the different regulatory elements based on their associated proteins, proposing a hierarchical arrangement with different enhancers and promoters in distinct subdomains.

Liu *et al.* also combined CAPTURE with chromosome conformation capture (3C) analysis for the sequencing of DNA associated with dCas9-bound loci, thereby identifying long-range intrachromosomal interactions. As expected, enhancer–promoter interactions were more frequent at expressed relative to repressed β-globin genes. The team also noted differences between the enhancers in the frequency of their interaction with promoters (suggesting distinct modes of action) and identified putative new regulatory elements based on their interactions with a known β-globin enhancer and the disruption of β-globin gene expression when deleted.

The investigators characterized a disease-associated intergenic region of the β-globin locus that, when deleted, results in persistence of fetal haemoglobin expression to adulthood. The high occupancy of various transcriptional regulators and long-range chromosomal interactions found across this intergenic region led the authors to propose that it serves as an important interaction hub to establish separate fetus-expressed versus adult-expressed domains across the β-globin locus.

To confirm that CAPTURE is applicable in other systems, Liu *et al.* delivered their expression constructs to mouse embryonic stem cells (ESCs) and showed using CAPTURE that, during differentiation to embryoid bodies, the properties of four ESC-specific enhancers changed. Interestingly, reductions in the frequency of long-range chromosomal contacts during differentiation preceded the changes in chromatin accessibility and histone acetylation, suggesting a temporal and functional hierarchy of gene regulation mechanisms, which the multi-omic nature of CAPTURE is well placed to dissect.

CAPTURE is thus a promising multilayered approach for understanding gene regulation mechanisms. It will be interesting to see the additional species, loci and disease contexts that CAPTURE can be applied to, as well as whether RNA sequencing (RNA-seq) of chromatin-associated RNAs will provide further insights beyond the proteins and DNA analysed in this study.

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ORIGINAL ARTICLE Liu, X. *et al.* In situ capture of chromatin interactions by biotinylated dCas9. *Cell* **170**, 1028–1043 (2017)