GENOMICS

Transcription triggers enhancer movement

CRISPR-based imaging of regulatory elements shows that their mobility increases as transcription is initiated.

The regulation of a gene's expression depends on regulatory elements that are often far removed on the linear genome. The laboratory of Joanna Wysocka at Stanford University has a long-standing interest in deciphering gene regulatory mechanisms that drive vertebrate development; one aspect of this is to understand how expression is related to the movement of enhancers and promoters in the 3D genome.

Her team wanted to use CRISPR-based imaging, the targeting of dCas9–GFP fusion proteins to loci of interest via single guide RNAs (sgRNAs), to follow the movement of regulatory regions in real time. Wysocka explained that current approaches suffer from low signal-to-noise ratios and inefficient delivery of a stoichiometric mix of sgRNAs to single cells. "We therefore focused on solving the guide RNA delivery problem," she said. Their CARGO (chimeric array of gRNA oligo) strategy first assembles multiple sgRNAs into a single vector by combining the variable seed regions of the sgRNAs with the fixed sgRNA scaffold prior to insertion into the vector backbone.

They used three CARGO arrays with 12 sgRNAs each to target dCas9–GFP to a 2-kilobase region immediately upstream of an enhancer cluster regulating *Fgf5*, a gene that contributes to the differentiation of embryonic stem cells into epiblast-like cells. As expected, the majority of cells showed a strong signal at two labeled loci per cell.

Tracking the *Fgf5* enhancer during differentiation, the researchers saw slow movement in mouse embryonic stem cells, where *Fgf5* is silent, but as the gene was being expressed during cell differentiation, the enhancer's movement increased. "Transcription-coupled increase in mobility of both enhancers and promoters was indeed the most surprising finding of our work," recalled Wysocka, but she added that in hindsight it seemed obvious that a molecular machine like polymerase II, which dissipates a large amount of energy, could stir up chromatin during transcription.

The researchers refer to the increased mobility of regulatory elements during transcriptionally active states as the 'stirring model'. These elevated dynamics will increase the likelihood of enhancer and promoter interactions and serve as a positive feedback loop for gene expression.

CARGO's application potential also includes the activation and repression of genes or the editing of multiple loci.

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RESEARCH PAPERS

Gu, B. *et al.* Transcription-coupled changes in nuclear mobility of mammalian cis-regulatory elements. *Science* **359**, 1050–1055 (2018).