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⤷ GENE REGULATION

A chromatin-based recruitment drive

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A cornerstone of synthetic biology and biological engineering is achieving regulatory control of genes of interest. Typically, this is attempted by placing binding sites for classic transcription factors upstream of genes. However, gene regulation is multilayered beyond transcription factor recruitment; thus, a new study has characterized how diverse chromatin regulators might provide a flexible and powerful way to regulate different aspects of gene expression.

Chromatin states in eukaryotic cells are modulated in various ways — including by DNA methylation, histone modifications and nucleosome remodelling — thus providing opportunities for 'fine-tuning' the regulation of gene expression. An emerging approach to assess the gene regulatory effects of specific chromatin regulator proteins is 'epigenome editing', in which chromatin regulators are fused to sequence-specific DNA-binding proteins to allow their recruitment to a chosen locus. Such a strategy has so far characterized only a few chromatin-modifying enzymes. So, Keung *et al.* took a systematic approach by generating a library of 223 yeast chromatin regulators fused to zinc-finger (ZF) DNA-binding proteins, although the system is potentially also applicable to the transcription activator-like effector (TALE) and CRISPR–Cas genome targeting systems. "Using this approach, we could rapidly interrogate a number of hypotheses of how chromatin and the proteins that modify it are able to regulate gene expression in both

time and space," explains senior author Ahmad Khalil.

The authors first tested their system by using different yeast strains that each contained one of the library constructs and a reporter construct in which a minimal promoter and a ZF-binding sequence are placed upstream of a fluorescent reporter gene. The findings functionally validated the known activating or repressive properties of the tested chromatin regulators.

As ZFs can be tailored to target different DNA sequences, combinatorial approaches involving multiple ZFs with different site specificities and fusion partners are possible. Keung *et al.* extended their method so that each chromatin regulatory fusion protein was co-recruited with a ZF fusion protein that contained the classic transcriptional activator protein VP16. The authors categorized the chromatin regulatory proteins into various classes of interactions with VP16 for reporter gene activation, ranging from antagonistic to synergistic. These data revealed that proteins mediating histone H3 lysine 4 trimethylation (H3K4me3) and nucleosome remodelling are the most synergistic and are thus likely to be the most potent for maximizing gene expression from synthetic constructs.

Next, the authors examined more complex spatial arrangements of chromatin regulator recruitment. They found that chromatin regulators typically had different (and sometimes opposite) effects when recruited upstream versus

downstream of reporter genes. In particular, chromatin remodellers activated expression when recruited upstream (probably through facilitating transcription factor recruitment) but repressed expression when recruited downstream (possibly by disrupting transcription elongation).

Finally, Keung *et al.* showed that recruitment of the Sir2 histone deacetylase or the Rph1 histone demethylase led to long-range gene silencing (which could be blocked by incorporating insulator DNA sequences into the reporter construct), and that recruitment of Sir2 resulted in histone deacetylation and gene repression that were stable through multiple cell divisions, even after Sir2 was no longer present.

Collectively, this study uncovers some of the regulatory logic of chromatin features that is likely to apply to both natural cell physiology and synthetic systems. Such knowledge should facilitate the design of custom gene expression systems and genetic circuits, including maximizing gene expression levels and achieving combinatorial, spatial, differential and temporal control of genes. "We plan to try to understand how chromatin can be engineered to robustly silence user-defined genes or entire loci and to establish stable 'memory' of environmental events recorded by cells," proposes Khalil as a possible application.

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