SYNTHETIC BIOLOGY

Complex regulatory control with CRISPR

Guide RNAs can serve as scaffolds to flexibly recruit different effector modules to specific genomic loci.

Gene regulation underlies much of the functional variation between cells. Methods to artificially control gene expression are therefore useful both to engineer desired cell types and to tease out the logic underlying gene regulatory networks. In recent work, Wendell Lim, at the University of California, San Francisco, and Lei Qi, at Stanford University, and colleagues, describe clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9–based gene regulation to for simultaneous activation and repression of genes in yeast and in human cells.

As previously shown, an enzymatically inactive form of the Cas9 nuclease can be fused to activator or repressor modules to effect regulation of a gene of interest. The Cas9 fusion is targeted, in each case, by a coexpressed guide RNA (gRNA) designed to recognize the desired genomic site. In their newest twist on CRISPR-Cas9–based gene regulation, Lim, Qi and colleagues turn the gRNA into a scaffold to recruit factors in addition to Cas9 to the target site.

They achieve this by adding sequences recognized by an RNA-binding protein to the 3′ end of the gRNA. Upon coexpression, the cognate RNA-binding protein fused to an effector module of interest—VP64 for gene activation or KRAB for repression, for example—is recruited to the target site. In both yeast and human, a gRNA bearing one of the well-characterized MS2, PP7 or com sequences specifically recruits the respective binding protein MCP, PCP or Com to the targeted gene. The resulting regulatory activity depends on which effector is fused to the recruitment module.

Not every combination of recruitment and effector module is active, and the magnitude of expression change varies in yeast and human. Nevertheless, for several combinations, the recruitment strategy achieves larger gene activation than Cas9-VP64 alone. Notably, the approach permits the simultaneous activation and repression of multiple genes; it is used to modulate three genes in a branched metabolic yeast gene regulatory network, achieving control over five distinct phenotypic outcomes.

Orthogonal control of gene regulation at multiple loci may be applicable to screens of more complex regulatory combinations than have so far been possible. And although the effectors used in the present study are either activators or repressors, future work may well recruit other modifiers, such as epigenetic modifiers, to a target locus.

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RESEARCH PAPERS

Zalatan, J.G. *et al.* Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. *Cell* **160**, 339–350 (2015).