

Repurposing Cas13 for Precise Translational Inhibition and Activation

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Project Goals: The Intrinsic Control for Genome and Transcriptome Editing in Communities (InCoGenTEC) project (funded under the Secure Biosystems Design initiative) seeks to develop technologies that foster the growth of our burgeoning bioeconomy, while maximizing biosafety, biosecurity, and biocontainment. We aim to expand our mechanistic and understanding of horizontal gene transfer within bacterial communities, and to harness synthetic mobile elements (SGEs) to create and deliver agile tools for transforming, controlling and detecting the genetic and biochemical state of bacteria. By improving our ability to engineer specific species (even non-isolatable ones) within bacterial communities in situ, we will enable better scientific understanding of microbial consortia and facilitate biotechnology applications that promote the growth of the bioeconomy while maintaining a paramount commitment to biosafety. We are harnessing the modular and programmable nature of CRISPR systems to create SGEs that enable more advanced manipulation of bacterial genomes, particularly in how they are expressed. Our team will then employ software to identify species-specific bacteriophages from near-neighbors and exploit them to deliver these modular SGEs into target species within a community.

Abstract:

Tools for synthetically controlling gene expression levels are a fundamental cornerstone of genetic engineering endeavors. While CRISPRi and CRISPRa technologies have been applied extensively towards control over transcription, comparatively little has been done in extending this control towards the translational level. Here, we employ CRISPR to modulate the rate of mRNA translation.

In the model prokaryote *E. coli*, approximately half of its genes are expressed in operons, where their transcription levels are tied to the surrounding genes encoded on the same mRNA. For this reason, state-of-the-art synthetic regulators like dCas9 and dCas12 are unable to selectively knock down a gene's expression without repressing genes downstream in the operon, prohibiting independent regulation of a large fraction of prokaryotic genes. We hypothesized that Cas13, an RNA-guided, RNA-targeting protein, might selectively block translation of a single gene on an mRNA molecule without impacting the expression of its operonic neighbors. To this end, our labs recently demonstrated that targeting catalytically inactive Cas13d (dCasRx) to the ribosome binding site of a fluorescent protein-encoding mRNA efficiently inhibits its translation in *E. coli*. We show here that dCasRx excels at targeted knockdown of genes in operons, selectively repressing individual fluorescent proteins in a three-gene synthetic reporter operon. We have also developed a genome-wide dCasRx guide RNA library in *E. coli*, consisting of ~150k pooled guides, to systematically determine design rules for efficient inhibition of translation and of non-coding RNA function. dCasRx represents the first application of a programmable RNA-binding protein for precise microbial gene regulation, holding great promise for functional genomics and synthetic gene regulation.

In tandem, we also demonstrate the application of Cas13 for enhancing translation rates. By targeting dCasRx to the start of the 5' untranslated region of RFP mRNA in *E. coli*, we demonstrate a 6.6-fold enhancement of gene expression. We further develop Cas13 as a translational activator by creating a novel variant dCasRx linked to the translation initiation factor IF3. We demonstrate dCasRx-IF3's ability to further enhance gene expression 16.0-fold above basal levels. Activation of translation is location-dependent, and we show dCasRx-IF3 maintains the ability to repress translation 7.4-fold when targeted to the RBS. Tuning experimental parameters resulted in 15.5-fold activation of RFP expression between dCasRx-IF3 and dCasRx strains, providing an insight into how dCasRx-IF3 can be further engineered and applied to enhance translation rates. We also demonstrate targeted upregulation of native β -galactosidase gene LacZ, highlighting dCasRx-IF3's versatility in application. Furthermore, we show that dCasRx-IF3 exhibits minimal fitness impacts and requires no additional host modification to influence gene expression.

Taken as a whole, this work outlines novel approaches for applying CRISPR for post-transcriptional control of mRNA translation rates to both inhibit and activate gene expression.

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