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Repurposing Cas13 for Precise Translational Inhibition and Activation

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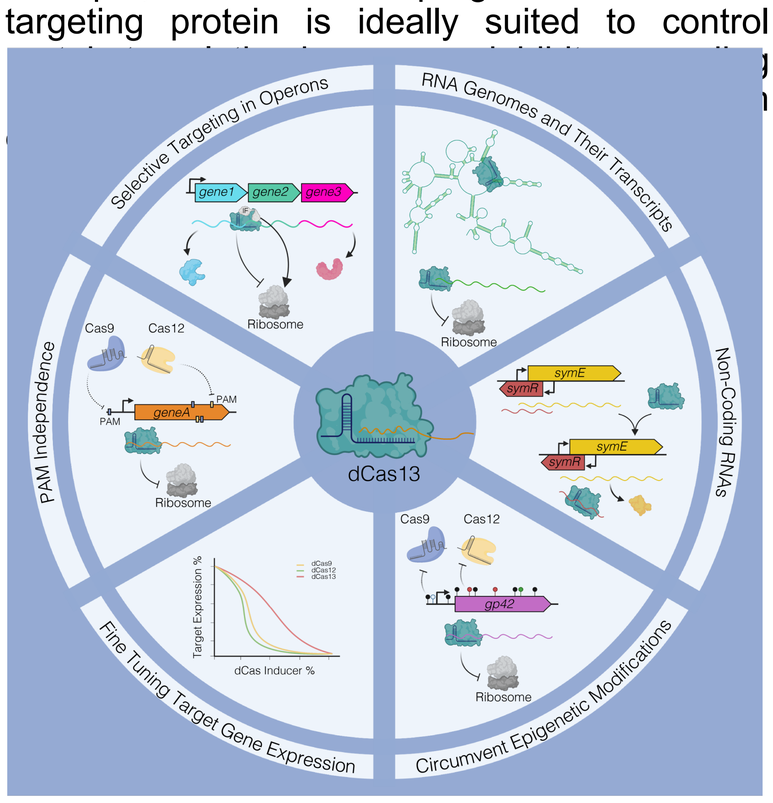
Abstract

Controlling gene expression is a fundamental cornerstone of genetic engineering endeavors. While CRISPRi and CRISPRa have been applied extensively towards transcriptional control, relatively little has been done to control translation. Here, we develop CRISPR-Cas13 tools for modulating the rate of mRNA translation. We target catalytically inactive Cas13d (dCasRx) to specific ribosome binding sites within an mRNA encoding three genes, and show this facilitates efficient, gene-specific translation inhibition in *E. coli*[1]. We also fuse dCasRx to translation initiation factors and demonstrate their ability to enhance gene expression 16-fold.

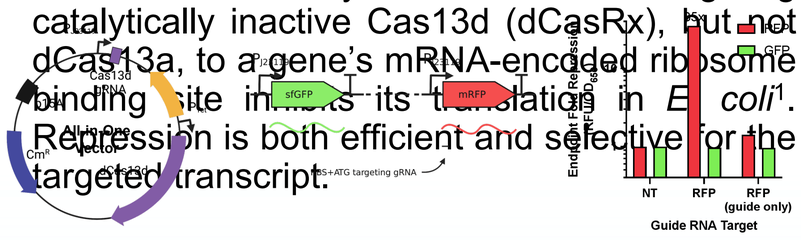
Background and Motivation

Programmable RNA-targeting proteins, like Cas13, possess many potential advantages relative to DNA-targeting CRISPR proteins. For example, an efficient programmable RNA-targeting protein is ideally suited to control



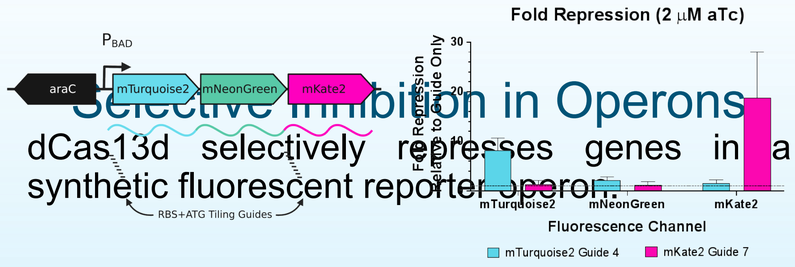
dCasRx Enables Translation Inhibition

Our labs recently showed that targeting catalytically inactive Cas13d (dCasRx), but not dCas13a, to a gene's mRNA-encoded ribosome binding site inhibits its translation in *E. coli*[1]. Repression is both efficient and selective for the targeted transcript.



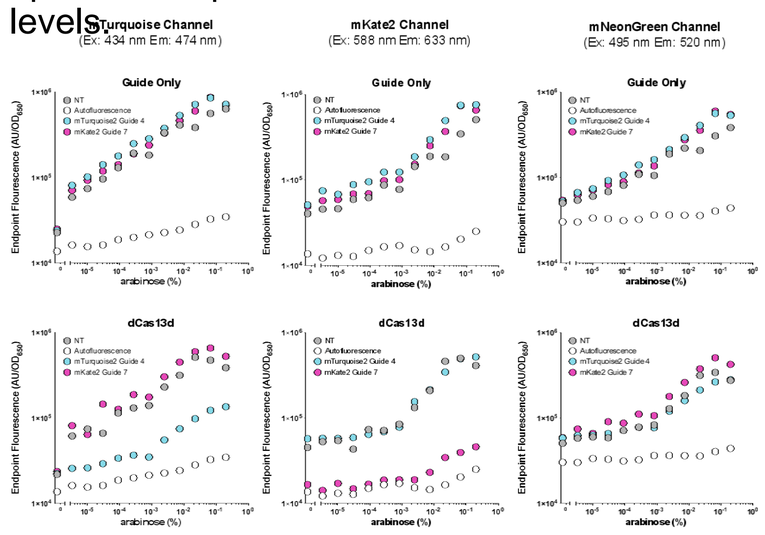
Selective Inhibition in Operons

dCas13d selectively represses genes in a synthetic fluorescent reporter operon.



Inhibiting Translation of Genes on Abundant mRNA Transcripts

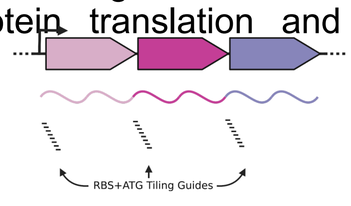
dCasRx efficiently and selectively inhibits translation of fluorescent proteins in a synthetic operon as reporter mRNA is induced to maximal levels.

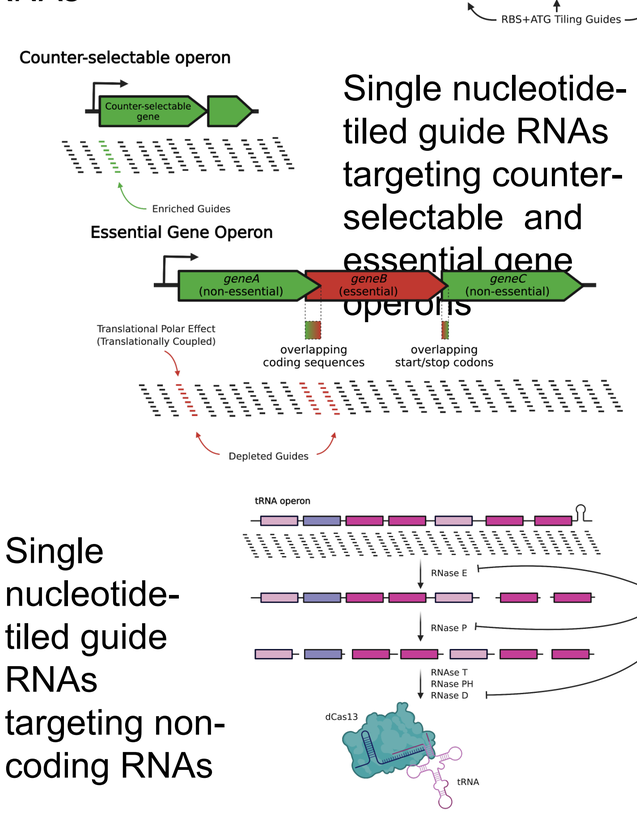


Guide Design Rules

We are screening genome-wide pooled guide RNA libraries in *E. coli* to systematically elucidate dCasRx guide design rules for efficient inhibition of protein translation and ncRNA function.

Coarsely-tiled, genome-wide RBS-targeting guide RNAs



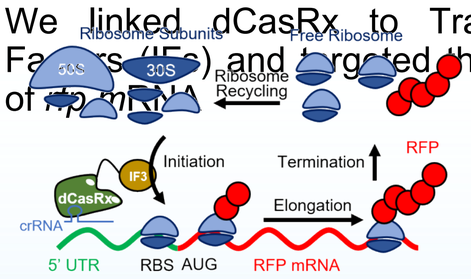


Single nucleotide-tiled guide RNAs targeting counter-selectable and essential gene operons

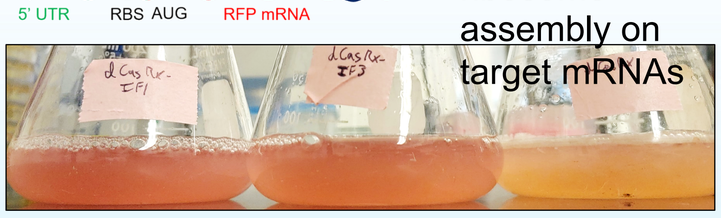
Single nucleotide-tiled guide RNAs targeting non-coding RNAs

Translation Activation

We linked dCasRx to Translation Initiation Factors (IFs) and targeted them to 5'UTR start of *rfp* mRNA.



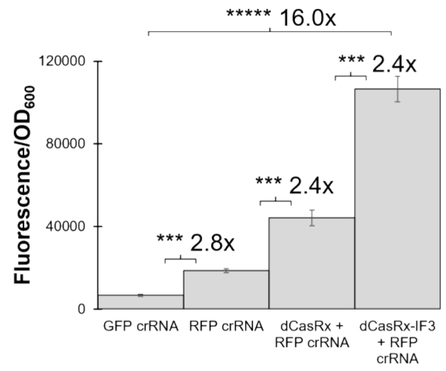
We predicted dCasRx-IF fusions would increase ribosome assembly on target mRNAs



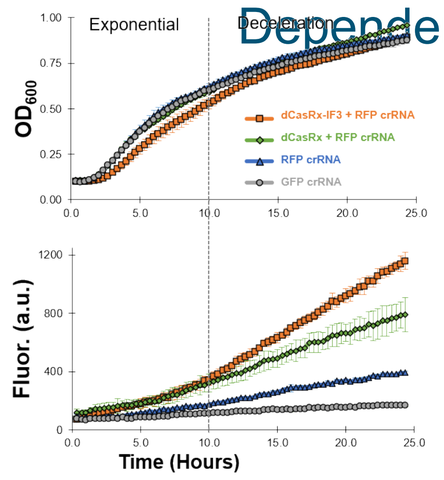
Fusions are visibly pinker, indicating activation

Component Effects on Activation

Targeting *rfp* with just crRNA increased expression[2]. This was enhanced when coupled with dCasRx, and even further enhanced with dCasRx-IF3.



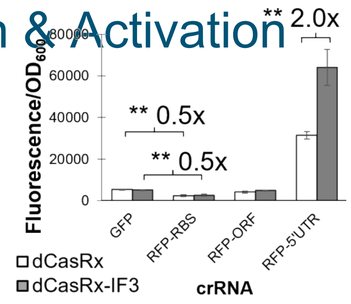
Activation and Phase-Dependency



dCasRx-IF3 slightly increased lag time (3.3hr) but had no impact on growth rates. Most of the IF3-specific effect emerged in late growth, suggesting a phase-dependent effect.

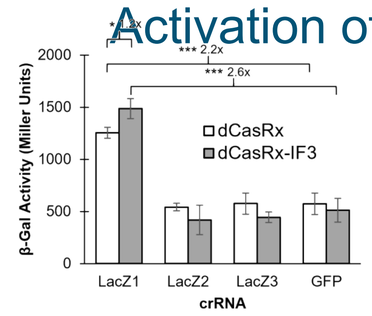
Target Location & Activation

Targeting the RBS reduced expression, while targeting inside the ORF had no impact, suggesting a location-dependent effect.



Activation of Native *lacZ*

Targeting *lacZ* mRNA (encoding β-Gal) with dCasRx enhanced expression, which was further enhanced by dCasRx-IF3.



Conclusions & Future Work

- dCasRx inhibits translation when targeted to the ribosome binding site and selectively represses genes encoded within the same operon.
- Translation from abundant transcripts is efficiently repressed by dCasRx.
- Genome-wide guide RNA libraries will illuminate design rules for efficient translational inhibition.
- Targeting start of 5' UTR with dCasRx enhances expression, and is augmented with fusion to IFs.
- Explore modifications to enhance IF effect, and import into a non-*E. coli* organism.

Acknowledgements

We thank Benjamin Adler for helpful discussions and advice.

References

1. Charles, "Engineering Improved Cas13 effectors for targeted post-transcriptional regulation of gene expression", bioRxiv, 2021
2. Isaacs, "Engineered riboregulators enable post-transcriptional control of gene expression", Nature Biotechnology, 2004

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NNSA National Nuclear Security Administration

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