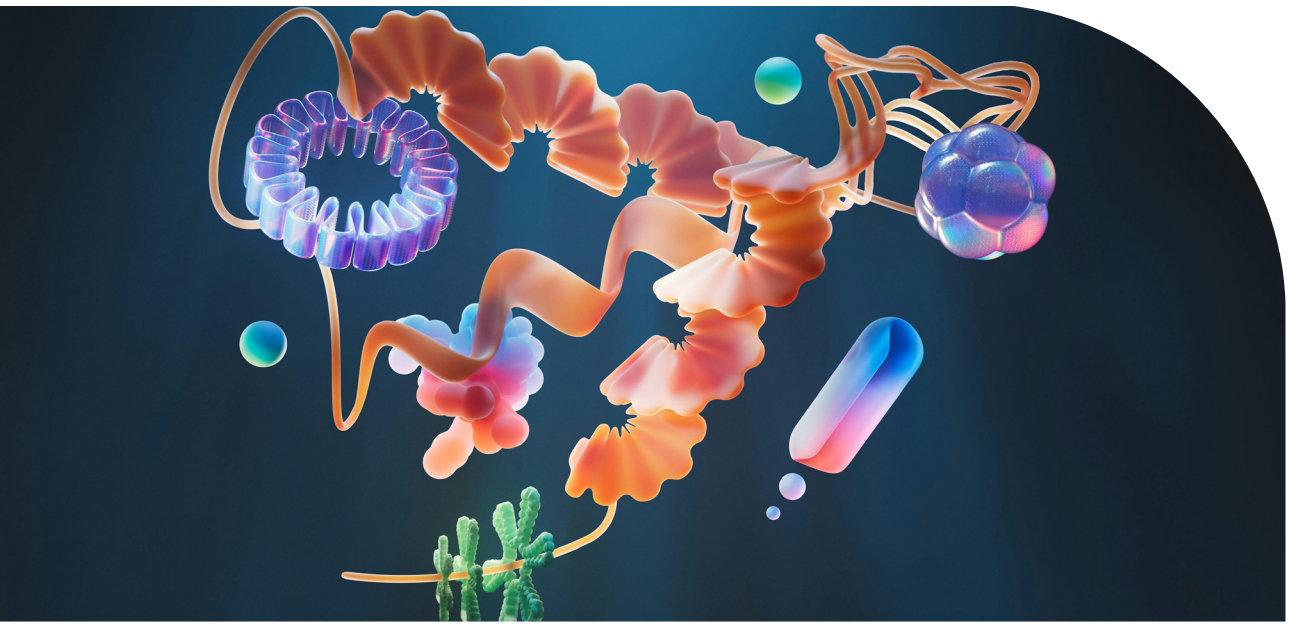
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Harnessing Type IV-A1 CRISPR for gene expression and plasmid replication control

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Case Study

Introduction

The CRISPR-Cas system is a powerful tool used by prokaryotes to defend against invading genetic elements such as phages and plasmids. Among these systems, Type IV-A1 CRISPR–Cas complexes have been found to be plasmid-encoded and capable of interfering with both gene expression and plasmid replication without cleaving DNA. This study investigates the mechanisms by which Type IV-A1 CRISPR-mediated interference (CRISPRi) regulates transcriptional activity in *Pseudomonas oleovorans* and *Escherichia coli* by using synthetic crRNAs. A key objective was to compare its gene repression activity with the widely used dCas9 system, particularly in downregulating extended genomic regions and plasmid targeting.

Materials and methods

Strains and growth conditions

The study utilized *P. oleovorans* DSM 1045 and *E. coli* BL21-AI, which were cultivated in LB media at 37°C. Where necessary, antibiotics were added to maintain selective pressure. The cells were engineered to express synthetic crRNAs targeting chromosomal and plasmid sequences through derivatives of the pSEVA424 vector and Duet vector derivates for *Pseudomonas* and *E. coli*, respectively. To induce crRNA expression, IPTG and arabinose were added at defined concentrations. Growth and fluorescence measurements were taken using a Magellan™ Infinite® 200 Pro plate reader.

RNA sequencing and data analysis

To gain insights into transcriptional changes, **Novogene's Illumina RNA sequencing services** were employed. This high-throughput sequencing approach enabled single-nucleotide resolution analysis of CRISPRi effects. The sequencing data was processed using FastQC for quality control, Cutadapt for trimming, Hisat2 for alignment, and DESeq2 for differential expression analysis. These tools helped identify and compare significant gene expression changes induced by Type IV-A1 and dCas9 CRISPRi.

Single-molecule microscopy (SMM)

To investigate crRNP localization and interactions with genomic and plasmid DNA, single-molecule microscopy was conducted. Fluorescently labeled Type IV-A1 crRNP complexes were tracked in live cells using a Nikon Ti2-Eclipse microscope equipped with nanoscopy modules. This technique allowed for precise visualization of crRNP diffusion behaviors and spatial distribution within bacterial cells in presence of chromosomal and target plasmids.

Results and discussion

CRISPRi effects on chromosomal gene expression

Type IV-A1 CRISPR–Cas activity was found to repress gene expression across an extended genomic region in *P. oleovorans*. Unlike dCas9, which exhibited localized interference limited to the immediate vicinity of its binding site, the Type IV-A1 system suppressed transcription across a broader range. RNA sequencing confirmed significant downregulation of histidine biosynthesis genes upon crRNP targeting. The presence of the CasDinG helicase was essential for extending interference beyond promoter-proximal regions. These findings were supported by Novogene's RNA sequencing services, which provided crucial transcriptomic insights.

Comparative analysis of Type IV-A1 CRISPRi and dCas9

A comparative analysis revealed key differences between Type IV-A1 CRISPRi and dCas9. While dCas9-mediated interference primarily affected downstream genes within operons, Type IV-A1 CRISPRi led to a more extensive inhibition, silencing entire operons. The broader effect was attributed to CasDinG's helicase activity, which facilitated the disruption of transcription elongation over a wider genomic region. Growth assays conducted in minimal media further confirmed that histidine auxotrophy induced by Type IV-A1 CRISPRi was stronger than that observed with dCas9-mediated repression.

Plasmid targeting in replication inhibition

Beyond chromosomal repression, Type IV-A1 CRISPR–Cas complexes also exhibited strong interactions with plasmids. Single-molecule microscopy demonstrated that crRNPs preferentially localized to high-copy plasmids, indicating a potential role in replication interference. When target sequences were present within plasmids, crRNPs accumulated at replication forks, as observed through tracking of DnaX-mScarlet fusion proteins. These findings suggest that Type IV-A1 CRISPR–Cas complexes act as physical roadblocks, effectively stalling plasmid replication and limiting its propagation.

Implications for biotechnological applications

The findings from this study have several important implications for biotechnology. The ability of Type IV-A1 CRISPR–Cas to silence genes across extended regions without DNA cleavage presents a promising avenue for gene regulation. This method could be particularly useful in synthetic biology applications where controlled gene expression is required. Additionally, the observed plasmid replication interference could be leveraged to develop new strategies for plasmid maintenance and propagation control. Given its broad reach, Type IV-A1 CRISPRi emerges as a potential alternative to dCas9 for long-range gene repression, offering enhanced efficiency in operon-wide regulation.

Conclusion

This study highlights the utility of Type IV-A1 CRISPRi as a powerful tool for transcriptional regulation and plasmid interference. The application of Novogene's RNA sequencing services provided high-resolution transcriptomic profiling, confirming the widespread repression effects of the system. Moving forward, further research could explore the use of this system for targeted gene knockdowns and plasmid control in biotechnology and synthetic biology, expanding its potential applications in microbial engineering and genetic circuit design.

Dr Mariana Sanchez-Londono



Dr. Mariana Sanchez-Londono is a dedicated researcher with over a decade of experience spanning RNA interference tools for agricultural pest management to uncover the mechanisms of Type IV-A1 CRISPR-Cas systems in bacteria. Her work has expanded the understanding of CRISPR-Cas beyond adaptive immunity, with a particular focus on transcriptome analyses and single-molecule microscopy techniques to study gene function at the molecular level.

Currently, she is a postdoctoral fellow at the Microcosm Earth Center, a joint initiative of the Max Planck Institute for Terrestrial Microbiology and Philipps-Universität Marburg, dedicated to promote early-career scientists with new research topics. Her research has led to first-author publications in high-impact journals such as *Nature Microbiology* and *Nucleic Acids Research*. She has also been recognized with multiple fellowships and grants, including the prestigious Christiane Nüsslein-Volhard-Stiftung grant for women in science with family responsibilities.

A passionate mentor, she has guided young scientists across Latin America, the U.S., Israel, and Germany. Looking ahead, she envisions her research at the intersection of ribonucleoproteins biology, epitranscriptome-based genetic tools, and AI-driven analysis, with implications for biotechnology and medicine.

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