Docket #: S18-496

Control of gene editing and gene expression via integrated anti-CRISPR proteins

Researchers at Stanford have developed methods for controlling CRISPR-based gene editing and gene regulation. CRISPR systems have been developed for gene editing and gene expression regulation in both prokaryotic and eukaryotic organisms. While CRISPR systems have many benefits, they also raise concerns due to the irreversible outcomes created by genome editing and the potential for use for malignant, unethical or illegal purposes. To ease these concerns, means to safely and reversibly control the activity of CRISPR tools are needed. Moreover, such means of control will provide new applications reliant on precisely tuning CRISPR activity. To help meet this need, the inventors have developed methods of using anti-CRISPR (Acr) proteins to stop CRISPR-mediated gene editing and regulation. This includes inhibition of gene editing, as well as inducible control of gene regulation. Moreover, it is possible to design robust gene circuits, such as those with pulse generators, within mammalian cells, thereby enabling novel methods for engineering genomes in a wide range of contexts.

Stage of research
The inventors have characterized Acr proteins and shown that a specific Acr is a potent regulator of (d)Cas9 activity in a wide variety of contexts and cell types. Additional development is ongoing.

Applications

- Research tool:
    - Inhibit CRISPR-mediated gene editing and regulation
    - Tunable control over CRISPR-based gene regulation
    - Construct intricate synthetic circuits within organisms

    - Design pulsatile gene expression to fine-tune expression levels and timing
- Biological security:
    - Generate cells that are immune to unlicensed gene editing applications
    - Preserve the integrity of yeast cell lines used in the production of sensitive materials such as toxic products or controlled substances
    - Safety net for counteracting CRISPR-based gene drives

Advantages

- Can prevent undesired CRISPR-based genome editing
- Allows for more advanced, dynamic, and adaptable control over Cas9 function
- Tunable inhibitor molecule- useful for probing biology in a wide range of contexts
- Small size of Acrs allows them to be easily incorporated in a wide range of contexts
- Remains highly efficient in inhibiting CRISPR activity when fused to other gene products
- Stable integration allows for permanent prevention of gene editing
- Potential to use dCas9 and Arcs to build dynamic pre-programed gene regulation circuits
- Can be engineered for multiple CRISPR system

Publications

- Nakamura M, Srinivasan P, Chavez M, Carter MA, Dominguez AA, La Russa M, Lau MB, Abbott TR, Xu X, Zhao D, Gao Y, Kipniss NH, Smolke CD, Bondy-Denomy J, Qi LS. Anti-CRISPR-mediated control of gene editing and synthetic circuits in eukaryotic cells. Nat Commun. 2019 Jan 14;10(1):194.

Patents

- Published Application: WO2020123512

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