(SD2021-181) Photo-activated Control of CRISPR-Cas9 Gene Editing

Tech ID: 32557 / UC Case 2021-Z08-1

ABSTRACT

Researchers from UC San Diego introduce RNA-CLAMP, a technology which enables site-specific and enzymatic cross-linking (clamping) of two selected stem loops within an RNA of interest. Intramolecular clamping of the RNA can disrupt normal RNA function, whereas subsequent photo-cleavage of the crosslinker restores activity. We applied the RNA-CLAMP technique to the single guide RNA of the CRISPR-Cas9 gene editing system. By clamping two stem loops of the single-guide RNA (sgRNA) with a photo-cleavable cross-linker, gene editing was completely silenced. Visible light irradiation cleaved the crosslinker and restored gene editing with high spatiotemporal resolution. Furthermore, by designing two photo-cleavable linkers which are responsive to different wavelength of lights, we achieved multiplexed photo-activation of gene editing in mammalian cells. Notably, although the Cas9-sgRNA RNP is not capable of DNA cleavage activity upon clamping, it maintained the capability to bind to the target DNA. The RNA-CLAMP enabled photo-activated CRISPR-Cas9 gene editing platform offers clean background, free choice of activation wavelength and multiplexing capability.

DESCRIPTION

RNA is one of the most important biomacromolecules in the living systems, manipulating a highly complex collection of functions which are critical to the regulation of numerous cellular pathways and processes. Being the cornerstone of biology’s central dogma, numerous approached has been developed to study and manipulate the functions of RNAs. However, compared to the study of proteins and DNAs/chromosomes, our understanding of RNA’s cellular function is significantly lacking. This is partially because of the transient nature of RNA molecule.The half-life of RNA is significantly shorter than DNA and protein. Besides, the detection of RNA suffers from low copy number as low as one copy per cell. Many creative methodologies have been developed in the past few decades to address this challenging question: how to label and manipulate cellular RNAs. Apart from non-covalent approaches, covalent RNA-modifying approaches have been challenging because of the difficulties in selectively modifying a single RNA of interest among the other RNAs in cellular conditions. Comparing to non-covalent interactions, covalent strategies provide an additional level of robustness in harsh cellular conditions.

CONTACT
Skip Cynar
scynar@ucsd.edu
tel: 858-822-2672.

OTHER INFORMATION

KEYWORDS

gene editing, photo activation, CRISPR, CRISPR-Cas9

CATEGORIZED AS

- **Biotechnology**
  - Genomics
- **Imaging**
  - Molecular
- **Research Tools**
  - Nucleic Acids/DNA/RNA

RELATED CASES

2021-Z08-1

Due to the covalent linkage, the conjugated functional groups will not be disassociated from the RNA of interest in most conditions. Besides, the low-molecular weight of small-molecule (< 2 kDa) minimize the perturbation of normal RNA functions. While many covalent RNA-modifying approaches have been developed, few methods allow for the selective labeling of a single post-transcriptional RNA among the complex cellular RNA pool.

SUGGESTED USES

Gene Editing

- This technology permits precise and rapid control of gene editing and will serve as a versatile tool in the future development of stimuli responsive gene editing technologies.
- Beyond gene editing, RNA-CLAMP provides a site-specific tool for manipulating the internal structure of functional RNAs

ADVANTAGES

- Optical control of gene editing in mammalian cells with high spatiotemporal resolution and multiplexing capability. The optical control of CRISPR-Cas9 offers non-invasive manipulation of gene editing with excellent spatiotemporal resolution.
- Minimzed cellular toxicity. By controlling the irradiation time period and light intensity, cellular toxicity can be minimized.
- Multiplexed activation of editing. Moreover, different wavelength of lights might be used to trigger gene editing at multiple genomic loci, enabling multiplexed activation of gene editing.

STATE OF DEVELOPMENT

See publication for demonstrated application of technology.

INTELLECTUAL PROPERTY INFO

This patent-pending technology in the United States is available for commercial development. UC San Diego welcomes licensing interest from companies in a position to commercialize this technology.

RELATED MATERIALS

- Zhang D, Liu L, Jin S, Tota E, Li Z, Piao X, Zhang X, Fu XD, Devaraj NK. Site-Specific and Enzymatic Cross-Linking of sgRNA Enables Wavelength-Selectable Photoactivated Control of CRISPR Gene Editing. J Am Chem Soc. 2022 Mar 16;144(10):4487-4495. doi: 10.1021/jacs.1c12166. Epub 2022 Mar 8. - 03/08/2022

University of California, San Diego
Office of Innovation and Commercialization
Tel: 858.534.5815
innovation@ucsd.edu

9500 Gilman Drive, MC 0910, ,
La Jolla,CA 92093-0910

https://innovation.ucsd.edu
Fax: 858.534.7345