



Increased efficiency of CRISPR-Cas9 genome editing (Dr. Wendy Gordon)

An approach that increases the efficiency of CRISPR/Cas-9 genome editing 30-fold by fusing Cas9 to an HUH endonuclease tethered to the desired donor DNA.

Applications

- Human gene therapy through introduction to stem cells
- Preventative gene therapy through editing of eggs
- Gene editing in plants
- Facilitates basic research in situations refractory to other editing techniques

Key Benefits & Differentiators

- **30-fold enhancement of CRISPR/Cas9-based gene editing:** HUH-endonucleases link donor DNA to Cas9, getting the desired template in proximity to the target loci and increasing editing efficiency.
- **Maintains Cas9 protein function:** Fusing HUH endonuclease to Cas9 preserves functionality of both proteins.
- **Facilitates multiple editing events simultaneously:** Variety of HUH proteins, each with distinct DNA binding allows the possibility of creating multiple Cas9-fusions each with unique target loci and DNA for incorporation.
- **Simple experimental design:** No need for modified DNA bases or additional steps for linking the donor DNA to the HUH-Cas9 fusion.

The unfulfilled potential of CRISPR-Cas9 gene editing

Genome editing using CRISPR-Cas9 has promised to deliver incredible capabilities for precise genome editing. While this approach is incredibly robust at targeting highly-specific sites in the genome to induce double-stranded breaks (DSB), these sites are most-frequently repaired by non-homologous end joining (NHEJ) that results in small, unspecific insertions or deletions of DNA. The highly desirable ability to introduce new, predetermined genetic information at these locations using homology-directed repair (HDR) has proven inefficient. Researchers at the University of Minnesota have developed an approach to enhance HDR efficiency by covalently linking the desired donor DNA to the Cas9-guide RNA complex through a fused HUH endonuclease. This effectively co-localizes (in both space and time) the donor DNA at the site of the DSB for incorporation, resulting in a 30-fold increase in HDR efficiency.

Getting all the pieces in the right place at the right time

HUH-endonucleases cut and bind to specific DNA sequences, resulting in a covalent link to the DNA (via tyrosine) in a rapid and stable manner. Furthermore, it has been shown that in a HUH-Cas9 fusion protein, both proteins retain activity. This fusion complex can be introduced to a guide-RNA directed to a desired genome location for editing, as well as ssDNA that includes the HUH target site and the preferred donor DNA sequences. The result of this combination is a Cas9 protein targeted to a specific location, tethered (by HUH) to the donor DNA for use in HDR. Even at low concentrations, this complex results in improved HDR efficiency in multiple cell

Technology ID

20160269

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types and at multiple target loci. The availability of multiple HUH proteins with different DNA recognition sequences also opens up the possibility of simultaneously incorporating multiple targeted editing events with different donor sequences in a single cell. Other strategies have attempted to co-deliver donor DNA with Cas9 machinery using either a biotin-avidin bridge or a SNAP tag. However, these approaches are more complicated experimentally, require incorporation of expensive and non-natural DNA bases or modifications, and cannot incorporate many editing complexes at once. HUH-Cas9 fusion proteins could facilitate progress in a variety of applications including plant editing in agriculture, human therapeutics (editing of stem cells and eggs) and a myriad of basic research scenarios.

Phase of Development

Proof of concept. Published work shows 30-fold enhancement of HDR in cells using HUH-Cas9 fusions in multiple editing assays (including repair, frameshift and in-frame insertions) in multiple cell types and at multiple loci.

Researchers

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[External Link](#) (cbs.umn.edu)

Publications

[*Increasing Cas9-mediated homology-directed repair efficiency through covalent tethering of DNA repair template*](#)

Communications Biology, (2018)1:54

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