

The Swiss Army knife of gene editing gets new control

September 5 2019, by Lori Dajose



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When researchers want to edit, activate, or silence a gene in any living organism, from bacteria to humans, they often turn to CRISPR/Cas9, a complex of RNA and protein that can act like a genetic Swiss Army knife.

Now, Caltech researchers have applied principles from the emerging

field of dynamic RNA nanotechnology to exert logical control over CRISPR/Cas9 within living cells. By engineering RNA strands to interact and thereby change shape in response to an RNA trigger sequence, the group demonstrates the ability to switch CRISPR/Cas9 from on to off and from off to on. The work suggests a path to confine manipulation of a gene only to specific organs, tissues, or [cell types](#) within an organism.

The work was done in the laboratory of Niles Pierce, professor of applied and computational mathematics and bioengineering, and is described in a paper published on June 4, 2019, in the journal *ACS Central Science*.

An organism's genome encodes complex biological processes that orchestrate the organism's development, maintenance, and repair. Different [genes](#) encode instructions for different cellular behaviors, such as growing, communicating, and dying. Controlling gene activity is a fundamental way to change the cell's behavior. Editing a gene, or, alternatively, turning it off or on in a given cell, provides biologists with a way to study the role of that gene, and likewise offers a promising avenue for doctors to treat disease.

Developed less than a decade ago, CRISPR/Cas9 technology has emerged as a game-changing tool for editing genomes and for controlling which genes are active and to what extent. The CRISPR/Cas9 complex is made up of two parts: Cas9, a protein that can edit genes; and the guide RNA (gRNA), a molecule that—as its name suggests—guides Cas9 to a target gene of choice. If desired, different variants of the Cas9 protein can be used to increase or decrease the level of activity of a target gene as well. In essence, a traditional gRNA executes the function "regulate gene Y," where the choice of target gene "Y" is specified by the sequence of the gRNA, and the kind of regulation (activate, silence, edit, and so on) depends on the choice of Cas9 variant.

One of the remarkable features of CRISPR/Cas9 technology is that these capabilities work on many organisms across the tree of life, whether they be fungi, plants, or birds. However, the versatility of this approach is limited by the fact that the gRNA is "always on"—that is, it executes its function independent of the cell type it is in. As a result, additional measures are needed to restrict regulation of the selected target gene "Y" to specific cells in a specific state. For example, in a scenario where some cells are diseased, it would be useful to restrict gene regulation to only that subset of cells.

An important signature of cell type and state is provided by the collection of RNA molecules present inside the cell. In principle, detection of an RNA sequence "X" (where X is a marker for a specific tissue type or disease state) could serve as a trigger to induce editing, silencing, or activation of an independent target gene Y. For the last 15 years, the Pierce Lab has pursued this vision, seeking to engineer RNA molecules that can detect an RNA trigger sequence X and then change shape to target an independent gene Y for regulation. CRISPR/Cas9 is one of several naturally occurring biological pathways to which this technology could be applied.

Now, led by graduate students Mikhail Hanewich-Hollatz and Zhewei Chen, a team of researchers has engineered guide RNAs that are conditional, changing shape in response to the presence or absence of an RNA trigger to switch between inactive and active conformations. As a result, these so-called conditional guide RNAs (cgRNAs) can execute logical functions such as "if X then not Y" (i.e., if the trigger RNA X is present, then silence the gene target Y) or "if not X then not Y." Unlike a traditional gRNA, cgRNAs are programmable at two levels, with the sequence of trigger X controlling where regulation occurs and the target-binding sequence controlling the subject of regulation (in other words, the identity of the target gene Y). In [bacterial cells](#), the team has been able to demonstrate both ON→OFF logic with initially active cgRNAs

that are turned off by an RNA trigger and OFFàON logic with initially inactive cgRNAs that are turned on by an RNA trigger. Moreover, in work led by research scientist Lisa Hochrein, they were able to successfully port one cgRNA mechanism from bacterial to mammalian cells, leveraging the portability for which CRISPR/Cas9 is renowned.

The hope is that cgRNAs might someday be applied to the treatment of disease, with RNA X as a disease marker and target Y as a therapeutic target, enabling selective treatment of diseased cells while leaving healthy cells untouched. Alternatively, the same logic could enable biologists to study the role of a gene of interest at a specific location and developmental stage within an embryo.

"There is still a long way to go to realize the potential of dynamic RNA nanotechnology for engineering programmable conditional regulation in living organisms, but these results with CRISPR/Cas9 in bacterial and mammalian [cells](#) provide a proof of principle that we can build on in seeking to provide biologists and doctors with powerful new tools," says Pierce.

More information: Mikhail H. Hanewich-Hollatz et al. Conditional Guide RNAs: Programmable Conditional Regulation of CRISPR/Cas Function in Bacterial and Mammalian Cells via Dynamic RNA Nanotechnology, *ACS Central Science* (2019). [DOI: 10.1021/acscentsci.9b00340](#)

Provided by California Institute of Technology

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