TOOLBOX

Molecular knob dials down gene activity

BY **ANN GRISWOLD**

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A new method harnesses the gene-editing system CRISPR to fine-tune gene expression in human cells. The tool, described 7 April in *Cell Stem Cell*, could help researchers probe the genetic roots of conditions such as autism[1].

CRISPR relies on a scissor-like enzyme called CAS9 to snip specific sites in a genome and **remove or replace DNA sequences**. But the system's effects are permanent and non-uniform, leaving some cells forever altered, whereas others escape edits altogether.

In the new tool, called CRISPR interference (CRISPRi), researchers strip CAS9 of its scissoring activity and stick the inactive enzyme to a site in the genome where it blocks the cell's DNA-reading machinery[2]. They can dial down a gene's expression to varying degrees by modifying CAS9's binding site and can also reverse the suppression.

The researchers compared CRISPR and CRISPRi head-to-head in a gene-silencing match. They first modified the CAS9 sequence for both systems so that it would stick to DNA only in the presence of the antibiotic doxycycline. They then used a virus to deliver the systems into human stem cells.

They also designed **guide RNAs** to escort CAS9 to a regulatory gene called NANOG that prevents stem cells from differentiating into other cell types. CRISPRi almost completely silences NANOG within one week of doxycycline treatment. Within a few days after that, the stem cells begin developing into mature cell types. By contrast, traditional CRISPR suppresses the gene in only 70 percent of cells after two weeks of doxycycline treatment.

The researchers got similar results when they compared the ability of the two tools to block OCT4, another protein that also prevents stem cell maturation. They tested CRISPRi's versatility in stem cells and mature cell types, using the new tool to silence nine other genes. After they removed doxycycline from the cells' diet, gene expression in the cells returned to normal.

The researchers were also able to dial down the silencing of each gene from 98 percent to 50 or 30 percent by designing guide RNAs that escort CAS9 to different sites in the genome, where it blocks the DNA-reading machinery from different angles. The versatile tool could also allow researchers to adjust gene activity at specific times during cellular development.

REFERENCES:

1. Mandegar M.A. *et al. Cell Stem Cell* **18**, 541-553 (2016) **PubMed**
2. Gilbert L.A. *et al. Cell* **159**, 647-661 (2014) **PubMed**