Broad-scale genome tinkering with help of an RNA guide

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Duke researchers have devised a way to quickly and easily target and tinker with any gene in the human genome. The new tool, which builds on an RNA-guided enzyme they borrowed from bacteria, is being made freely available to researchers who may now apply it to the next round of genome discovery.

The new method also has obvious utility for gene therapy and for efforts to reprogram stem or adult cells into other cell types – for example, to make new neurons from skin cells.

"We have the genome sequence and we know what all the parts are, but we are still in need of methods to manipulate it easily and precisely," says assistant professor Charles Gersbach, of Duke's Pratt School of Engineering and the Duke Institute for Genome Sciences & Policy. "That's where this engineering tool comes in."

Gersbach's team had already been in the business of tinkering with the genome using specially engineered proteins, but the process was difficult and slow. It was hard to imagine how to scale it up for the investigation of hundreds or even thousands of genes in the way genome scientists really wanted to do. "That's where the conversation always broke down," he says.

Then, he and post-doctoral researcher Pablo Perez-Pinera found out about an RNA-guided protein called Cas9 found in a Streptococcus bacteria. The bacteria rely on Cas9 as part of an adaptive immune system to defend themselves against infection by viruses, cutting out a piece of the viral DNA and inserting it into their own genome for recognition of future infection. Other scientists then showed that those immune system components could function inside human cells.

Gersbach's team recognized the RNA-guided nature of this system as a potential game-changer for the gene engineering work they do.

In the study now reported in *Nature Methods* on July 25, Gersbach and his colleagues modified Cas9 to turn genes on rather than cut them. They showed that their tool could turn on very specific genes in human cells. They went on to demonstrate use of the tool to modify targets of interest for fighting inflammation and activating gene networks for making neurons, muscle cells or stem cells. They showed they could induce a gene known to alleviate symptoms of sickle cell disease, too.

In other words, it works, and it works on genes that matter from a clinical perspective. In principle, the RNA-guided tool could be used to modify or influence any gene anywhere in the genome.

Gersbach now hopes to apply the new tool along with collaborators in the IGSP to investigate the functions of thousands of sites across the genome. With tissue engineer Farshid Guilak, a professor of engineering and orthopaedic surgery, he will continue to work on its application in the fight against inflammatory and autoimmune diseases such as arthritis.

"This simple and versatile tool makes it easy for anyone to do this," Gersbach says.

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