Researchers develop faster method of engineering zinc-finger nucleases

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A team led by Massachusetts General Hospital (MGH) researchers has developed a faster way to engineer synthetic enzymes that target specific DNA sequences for inactivation, repair or alteration. The report from the MGH Molecular Pathology Unit, being published online in Nature Methods, describes a highly effective but less labor-intensive way to generate powerful tools called zinc-finger nucleases (ZFNs).

"With our approach, called context-dependent assembly, any scientist can use either standard molecular biology techniques or commercial DNA synthesis to design ZFNs for their target gene of interest," says J. Keith Joung, MD, PhD, associate chief for Research in MGH Pathology, the study's senior author. "ZFNs are broadly applicable, powerful tools for manipulating the genomes of cells from various organisms – including humans – and may provide a way to efficiently correct gene mutations responsible for human disease, avoiding problems resulting from the imprecise nature of current gene therapy approaches using viral vectors."

Most human transcription factors that control whether a genetic signal is translated into a protein bind to specific DNA sequences using peptides called zinc fingers. Zinc-finger nucleases are synthetic "designer" proteins combining a zinc-finger domain, engineered to bind a particular DNA sequence, with an enzyme that breaks both DNA strands at the targeted site. While ZFNs have great potential, creating the customized proteins has been challenging.

In the simplest approach, called modular assembly, individual peptides are linked together like beads on a string to create a multi-finger protein theoretically able to recognize long DNA segments. Joung and others have shown that, in practice, modular assembly has a very low success rate for creating multi-finger proteins. This high failure rate is most likely due to "context-dependent" effects that individual zinc fingers can have on the DNA-binding activities of their neighboring fingers. Assembling peptides that don't work well together would be like trying to put together jigsaw puzzle pieces that don't fit.

In 2008, Joung and colleagues at the University of Minnesota and other institutions, members of the Zinc Finger Consortium, reported developing a method called OPEN (Oligomerized Pool ENgineering), which takes these context-dependent effects into account. But although OPEN works well, it can be labor intensive and extremely time consuming – requiring up to a year for a lab to establish the technology and two months of work to generate desired ZFNs. To address these limitations, the MGH research team has assembled an extensive archive of zinc fingers known to work well when positioned together – in essence puzzle pieces that already have been put together. Using this context-dependent method, the investigators were able to assemble dozens of ZFNs in as little as four days.

"With this archive in hand, any researcher can easily generate their own ZFNs in less than a week, and no special expertise is needed," Joung explains. "In addition to being much faster, context-dependent assembly can generate large numbers of ZFNs simultaneously, which is hard to do with OPEN because it is more labor intensive." As was the case with OPEN, the Joung lab and the Zinc Finger Consortium will make the software and reagents required to practice context-dependent assembly available to all academic laboratories.

"One of the holy grails of genetics is the ability to make targeted changes

to individual genes," says Laurie Tompkins, PhD, who over sees genetics grants at the National Institute of General Medical Sciences, one of the National Institutes of Health and a major supporter of this study. "Dr. Joung and his colleagues have developed an extraordinarily simple, efficient strategy for using zinc finger technology to swap out altered versions of genes for normal ones – or vice versa – providing basic scientists and clinicians alike with a broadly applicable research tool."

Adds Joung, an associate professor of Pathology at Harvard Medical School, "At this point, I believe that context-dependent assembly will have the biggest impact on researchers using ZFNs to genetically manipulate model organisms, possibly even models developed from pluripotent stem cells. Other big impacts should be enabling researchers to create knockout mutations in a large series of genes involved in a common pathway or related to a specific disease and to use ZFNs to create comprehensive collections of mutants for every gene in an organism." Joung is also a member of the MGH Center for Computational and Integrative Biology and Center for Cancer Research.

The challenges posed to scientists interested in using ZFNs in their investigations were described in an article in the Fall 2010 issue of the MGH-sponsored magazine Proto, which can be accessed at protomag.com/assets/zinc-fingers-entry-fee .

More information: Jeffry Sander, PhD, of the MGH Molecular Pathology Unit is lead author of the *Nature Methods* report.

Provided by Massachusetts General Hospital

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