Consortium develops new method enabling routine targeted gene modification

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A multi-institutional team led by Massachusetts General Hospital (MGH) investigators has developed a powerful new tool for genomic research and medicine – a robust method for generating synthetic enzymes that can target particular DNA sequences for inactivation or repair. In the July 25 issue of *Molecular Cell,* the researchers describe an efficient, publicly available method to engineer customized zinc-finger nucleases (ZFNs), which can be used to induce specific genomic modifications in many types of cells.

"Recent work has shown that ZFNs can alter genes with high efficiency in cells from plants or model organisms like fruitflies, roundworms and zebrafish, and in human cells," says J. Keith Joung, MD, PhD, of the MGH Molecular Pathology Unit, the paper's senior author.

"However, a significant bottleneck has been the lack of access to an effective method for generating the customized DNA-binding domains needed to guide ZFNs to their target sites. Our method will enable academic researchers to rapidly create high quality ZFNs for genes of interest and will stimulate use of this technology in biological research and potentially gene therapy."

Zinc-finger peptides, which bind to DNA, occur naturally in many important proteins that regulate or otherwise interact with DNA. Zinc-finger nucleases are constructed from synthetic "designer" zinc-finger domains targeted to a specific genetic sequence and another protein segment that breaks both DNA strands within the binding site. Currently

available methods for generating ZFNs are either inefficient or involve constructing and analyzing huge libraries of zinc-finger peptides, a task that exceeds the capabilities of all but a handful of laboratories in the world.

First author Morgan L. Maeder of the Joung lab led an effort by researchers from six institutions that demonstrated how this new method (termed OPEN for Oligomerized Pool ENgineering) can rapidly generate ZFNs that induce alterations at sites in three biologically important human genes and a plant gene. ZFNs made by the new OPEN method – which utilizes a new archive of reagents that will be made publicly available by the Zinc Finger Consortium – were so efficient that they could modify as many as four copies of a gene in human cells and two copies in plant cells.

"Our study provides the first evidence that ZFNs can make specific changes in plant genes with high efficiency and opens a new avenue for plant genetic modification," says Daniel Voytas, PhD, of the University of Minnesota, whose lab conducted the plant cell experiments. Recently relocated from Iowa State University, Voytas and his team are interested in modifying plant genes for crop improvement.

"With the development of OPEN, many more academic labs will be able to construct, test and use ZFNs in their biological research projects," adds Joung. "OPEN should also stimulate additional research into the potential application of ZFNs for gene therapy of single-gene disorders, such as sickle cell anemia and cystic fibrosis." Joung's lab has already begun to explore ways to further simplify the OPEN method so that it can be performed more quickly and for a larger number of gene targets at once. He is an assistant Professor of Pathology at Harvard Medical School and director of the Molecular Pathology Unit at MGH.

Source: Massachusetts General Hospital