

## A New Technique for Modifying Plant Genes

*Editor's Note: In my previous columns, we have examined GMO plants and discussed the techniques used to facilitate traits such as herbicide resistance. These developments most frequently involve transfer of foreign DNA into the plant genome. Researchers at the University of Minnesota and Massachusetts General Hospital have used a genome engineering tool they developed to make a model crop plant herbicide-resistant without significant changes to its DNA. "It's still a GMO but the modification was subtle," said Daniel Voytas, lead author and director of the U of M Center for Genome Engineering. "We made a slight change in the sequence of the plant's own DNA rather than adding foreign DNA. This is the first real advance in technology to genetically modify plants since foreign DNA was introduced into plant chromosomes in the early 1980s," Voytas said. "It could become a revolutionary tool for manipulating plant, animal and human genomes."*

The new approach has the potential to help scientists modify plants to produce food, fuel and fiber sustainably while minimizing concerns about genetically modified organisms

For the study, the researchers created a customized enzyme called a zinc finger nuclease (ZFN) to change single genes in tobacco plant cells. The altered cells were then cultured to produce mature plants that survived exposure to herbicides.

Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target desired DNA sequences which enable zinc-finger nucleases to target unique sequence within a complex genome. By taking advantage of endogenous DNA repair machinery; these reagents can be used to precisely alter the genomes of higher organisms.

### DNA-cleavage domain

The non-specific cleavage domain from the type II restriction endonuclease FokI is typically used as the cleavage domain in ZFNs. This cleavage domain must dimerize in order to cleave DNA and thus a pair of ZFNs are required to target non-palindromic DNA sites. Standard ZFNs fuse the cleavage domain to the C-terminus of each zinc finger domain. In order to allow the two cleavage domains to dimerize and cleave DNA, the two individual ZFNs must bind opposite strands of DNA with their C-termini a defined distance apart. The most commonly used linker sequences between the zinc finger domain and the cleavage domain requires the 5' edge of each binding site to be separated by 5 to 7 base-pairs.

### DNA-binding domain

The DNA-binding domains of individual ZFNs typically contain between three and six individual zinc finger repeats and can each recognize between 9 and 18 base-pairs. If the zinc finger domains are perfectly specific for their intended target site then even a pair of 3-finger ZFNs that recognize a total of 18 base-pairs

can theoretically target a single locus in a mammalian genome.

Various strategies have been developed to engineer Cys2His2 zinc fingers to bind desired sequences. These include both "modular assembly" and selection strategies that employ either phage display or cellular selection systems.

The most straightforward method to generate new zinc-finger arrays is to combine smaller zinc-finger "modules" of known specificity. The most common modular assembly process involves combining three separate zinc fingers that can each recognize a 3 base-pair DNA sequence to generate a 3-finger array that can recognize a 9 base-pair target site. Other procedures can utilize either 1-finger or 2-finger modules to generate zinc-finger arrays with six or more individual zinc fingers. The main drawback with this procedure is the specificities of individual zinc fingers can overlap and can depend on the context of the surrounding zinc fingers and DNA. Without methods to account for this "context dependence", the standard modular assembly procedure often fails unless it is used to recognize sequences of the form (GNN)<sub>3</sub>N.

Numerous selection methods have been used to generate zinc-finger arrays capable of targeting desired sequences. Initial selection efforts utilized phage display to select proteins that bound a given DNA target from a large pool of partially randomized zinc-finger arrays. More recent efforts have utilized yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells. A promising new method to select novel zinc-finger arrays utilizes a two-hybrid system and has been dubbed "OPEN" by its creators. This system combines pre-selected pools of individual zinc fingers that were each selected to bind a given triplet and then utilizes a second round of selection to obtain 3-finger arrays capable of binding a desired 9-bp sequence. This system was developed by the Zinc-Finger Consortium as an alternative to commercial sources of engineered zinc-finger arrays.

### Applications

Zinc finger nucleases have become useful reagents for manipulating genomes of many higher organisms including *Drosophila melanogaster*, *Caenorhabditis elegans*, tobacco, rats, various types of mammalian cells, and zebrafish. An ongoing clinical trial is evaluating ZFNs that disrupt the CCR5 gene in CD4+ human T-cells as a potential treatment for HIV/AIDS.

The ability to precisely manipulate the genomes of plants, animals and insects has numerous applications in basic research, agriculture, and human therapeutics. Using ZFNs to modify endogenous genes has traditionally been a difficult task due mainly to the challenge of generating zinc finger domains that target the desired sequence with sufficient specificity. Improved methods of engineering zinc finger domains and the availability of ZFNs from a commercial supplier now put this technology in the hands of increasing numbers of researchers.

Garry Smith can be reached at [garrypatsysmith@msn.com](mailto:garrypatsysmith@msn.com)