Primer: genome editing with engineered nucleases

A brief description of tools for targeted cleavage and tailored modification of genomes is presented.

Genomes are ordered and controlled in large part by sequence-specific DNA binding proteins. The principles underlying such binding are increasingly being exploited to engineer tools for targeted genome editing. This essentially involves two steps. First, a nuclease is engineered to cleave the desired sequence in the genome, creating a double-strand break. Second, the cell's ability to resolve the break via one of two well-conserved repair pathways is harnessed to generate a modification of choice at that genomic location.

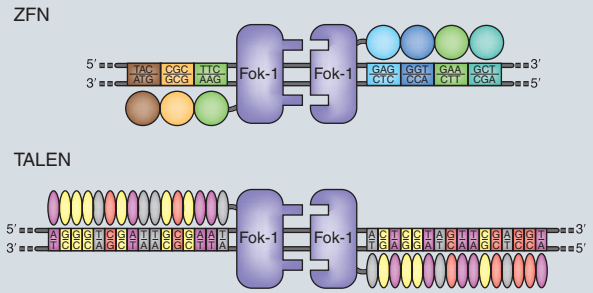
Making the cut

There are three principal classes of engineered nucleases.

Zinc finger nucleases (ZFNs) consist of a DNA-binding domain, derived from zinc-finger proteins, linked to the nuclease domain of the restriction enzyme Fok-1. Like their parent nuclease, ZFNs must dimerize to cleave DNA. The DNA-binding domain, which can be designed (in principle) to target any genomic location of interest, is a tandem array of Cys_2His_2 zinc fingers, each of which recognizes three nucleotides in the target DNA sequence. By linking together multiple fingers (the number varies: three to six fingers have been used per monomer in published studies), ZFN pairs can be designed to bind to genomic sequences 18–36 nucleotides long. When two ZFN monomers bind, in inverse orientation, with an optimal spacing of 5–7 nucleotides, the resulting dimeric nuclease cleaves the DNA between the binding sites.

Transcription activator–like effector nucleases (TALENs) have an overall architecture similar to that of ZFNs, with the main difference that the DNA-binding domain comes from TAL effector proteins, transcription factors from plant pathogenic bacteria. The DNA-binding domain of a TALEN is a tandem array of amino acid repeats, each about 34 residues long. The repeats are very similar to each other; typically they differ only at two positions (amino acids 12 and 13, called the repeat variable di-residue, or RVD). Each RVD specifies preferential binding to one of the four possible nucleotides, meaning that each TALEN repeat binds to a single base pair. TAL effector DNA binding is mechanistically less well understood than that of zinc-finger proteins, but their seemingly simpler code could prove very beneficial for engineered-nuclease design. TALENs also cleave as dimers, have relatively long target sequences (the shortest reported so far binds 13 nucleotides per monomer) and appear to have less stringent requirements than ZFNs for the length of the spacer between binding sites.

Meganucleases are a subset of the homing endonucleases found widely in microbial (and the corresponding viral) genomes. They cleave DNA with very high specificity at long genomic target sites. If naturally occurring enzymes of this class are to be used for tailored genome modification, the target site must first be introduced into the genomic location of interest. Extending the application of meganucleases to target endogenous genes requires the development of variant enzymes, which has been achieved in some cases.



ZFNs and TALENs. Schematic (not to scale) of a ZFN pair (top) and a TALEN pair (bottom) to illustrate the DNA-binding principles of these enzymes. Image modified from *Nat. Methods* **8**, 53–55 (2011).

Fixing the cut

Cells invariably mend breaks in their DNA. Once an engineered nuclease has cleaved the genome, DNA-repair pathways are activated. Broadly speaking, repair occurs in one of two ways: the cleaved ends may be joined back together in an error-prone process called nonhomologous end-joining (NHEJ), or an exogenous template may be used to repair the break by homology-directed repair (HDR). Repair by NHEJ generates small insertions or deletions that can result in gene mutation or even complete knockout. In contrast, to make controlled sequence changes—to correct an existing mutation, for instance—HDR must be harnessed. This is achieved by introducing homologous DNA that includes the desired change into the same cell as the nuclease; this DNA serves as a template for repair by homologous recombination.

A menagerie of edited genomes

Both NHEJ and HDR are operational in essentially all species. Genome editing after targeted cleavage by an engineered nuclease should therefore be a very general approach for reverse genetics. Indeed, first with ZFNs, and increasingly with TALENs, many organisms have proven amenable to manipulation with these tools. Mutation of endogenous genes has been achieved in rat, mouse, fish, fly, frog, pig, sea urchin, worm, human cells and more than one species of plant. Genes have been added into specific loci, endogenous genes have been tagged and gene mutations have been corrected, in more than one species. Meganucleases have also been used to mutate endogenous loci in human cells. It should be noted, however, that genome editing with engineered nucleases is not yet an entirely solved problem: challenges remain with delivery, specificity, efficient design of robust tools and more controlled access to all of the genome.

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