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GENETICS

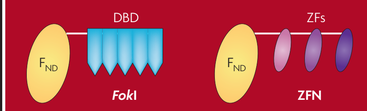
Applications of zinc finger nucleases

Dana Carroll

ZFNs have emerged as powerful tools for the directed modification of genomic DNA sequences in many different organisms. The key to this capability is the ability to design ZFNs to target specific sequences. This in turn is a property of the DNA-recognition domain of these proteins, which is made up of modular DNA-binding units called zinc fingers. New finger combinations can be constructed to recognize new gene targets, and DNA cleavage occurs specifically at the designed site. The result of cleavage is either the introduction of new mutations specifically at the target or the exchange of the existing sequence for one provided by the experimenter. This approach has been used effectively in a number of experimental organisms. It holds great promise for the directed modification of crop plants and for human gene therapy.

Origin and properties

*Fok*I endonuclease



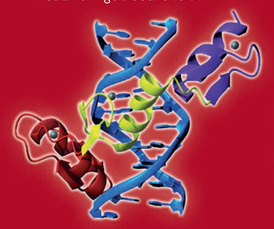
ZFNs consist of a non-specific cleavage domain from the restriction enzyme *Fok*I linked to a set of DNA-binding zinc fingers (as shown). Natural *Fok*I is a two-domain protein that binds a specific 5-bp DNA sequence and cuts a distance away on the two strands. Chandrasegaran and colleagues[1] showed that new cleavage specificity could be conferred by replacing the natural DNA-binding domain of *Fok*I by one with a different recognition specificity. In ZFNs, the site of DNA cleavage is determined by the recognition specificity of the zinc fingers.

Zinc fingers

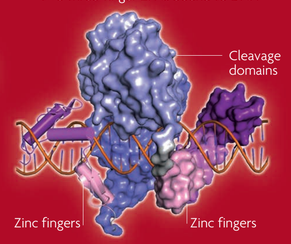
Each zinc finger contacts and recognizes primarily 3 bp of DNA.

One zinc finger

Three zinc fingers bound to DNA



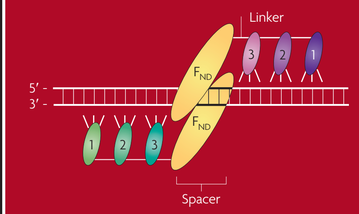
Three-dimensional representation of two three-finger ZFNs bound to DNA



Cys_2His_2 zinc fingers are small protein modules of approximately 30 amino acids and a single zinc atom coordinated by two cysteine and two histidine residues. When binding to DNA, each finger principally contacts 3 bp, using side chains from three amino acids. Consecutive fingers bind consecutive DNA triplets. It was recognized by Carl Pabo[2] and others that the regular, repetitive nature of DNA recognition by zinc fingers might allow the design of new fingers to bind many different DNA sequences. The first two images are reproduced, with permission, from REF. 3 © (2001) Annual Reviews.

Dimerization and specificity

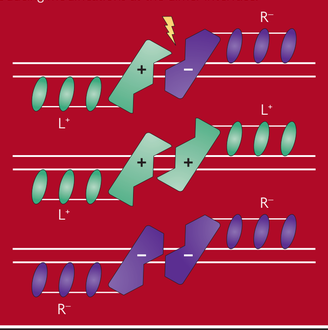
Like natural *Fok*I, ZFNs must dimerize to cut DNA. As the natural dimer interface is quite weak, cleavage requires two sets of zinc fingers (1, 2 and 3), each linked to one cleavage domain (F_{ND}), which is bound to nearby sites on the two DNA strands.



The optimum spacer between zinc finger-binding sites is 5 or 6 bp when the linker between the protein domains is quite short.

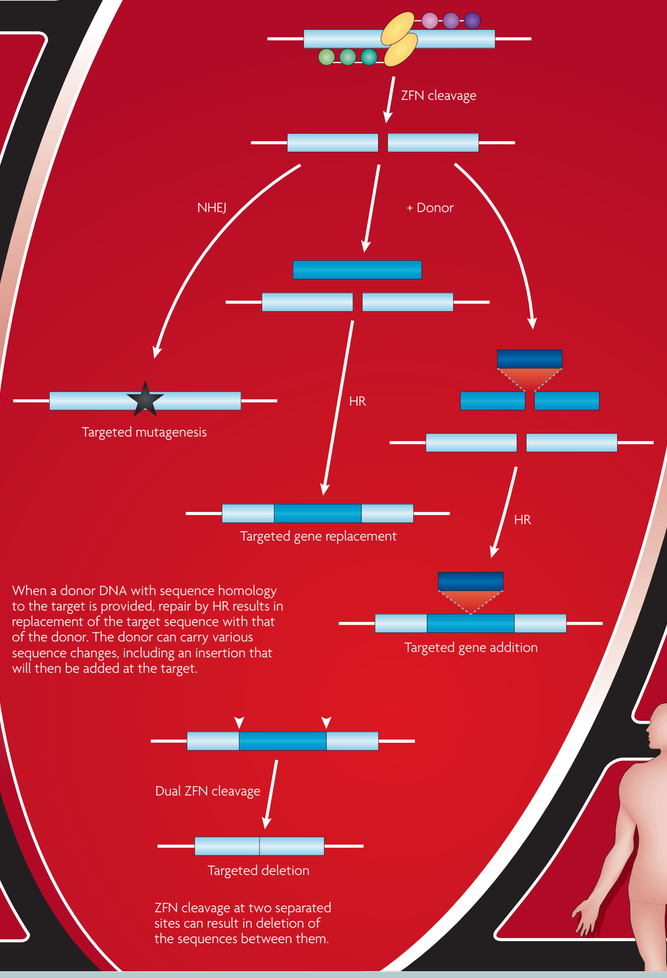
Although each zinc finger binds 3 bp of DNA, they are not always perfectly specific – therefore, a finger may bind several different triplets.

The specificity of ZFN cleavage can be enhanced by adding more fingers. Proteins with up to six fingers have been used successfully. Specificity can also be improved by preventing cleavage by two molecules of a single ZFN while permitting cleavage by the designed heterodimer. This has been accomplished by introducing modifications at the dimer interface.



Gene targeting

A pair of ZFNs designed to target a genomic sequence will make a break in both DNA strands specifically at that site. DNA DSBs are potentially lethal and therefore cells have several pathways for repairing breaks and restore intact DNA. There are two major DSB repair pathways: copying of information from the corresponding chromosome by HR, or simply joining the broken ends in a process called NHEJ, which often results in new mutations at the target sequence. These can include small or large deletions and insertions, or base substitutions.



When a donor DNA with sequence homology to the target is provided, repair by HR results in replacement of the target sequence with that of the donor. The donor can carry various sequence changes, including an insertion that will then be added at the target.

ZFN cleavage at two separated sites can result in deletion of the sequences between them.

Applications

The functions of particular genes can be studied in organisms that can be manipulated experimentally. ZFNs make it possible to introduce DNA sequence changes into individual genes and evaluate the effects of these changes. The flexibility in sequence recognition offered by combinations of zinc fingers means that essentially any gene can be targeted uniquely.

This approach has been applied to a number of different organisms that are used in various biological studies; in addition, targeted disruptions have recently been reported in sea urchins and silkworms. In future, ZFN technology should be applicable to virtually any organism. The main challenge in each case will be finding effective methods for delivering the ZFNs and donor DNAs.

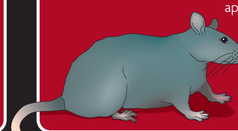
Drosophila melanogaster
The first application of ZFNs to a genomic sequence was in the fruitfly, an organism that is very popular for genetic studies. Both gene disruption by NHEJ and gene replacement by HR have been achieved at frequencies of around 10%.

Caenorhabditis elegans
This small nematode worm is widely used because of its simple anatomy and rapid development. To date, only somatic gene disruptions have been achieved with ZFNs.

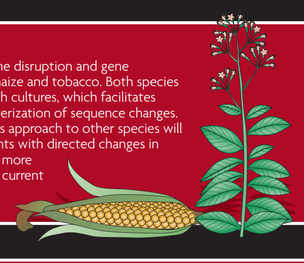
Danio rerio
Although popular as a model vertebrate, no method for making targeted mutations was available for zebrafish. ZFN-induced gene disruptions have been made in many targets at frequencies of around 10%. This has greatly facilitated genetic analysis and the ZFN approach is now widely used.

Arabidopsis thaliana
This small flowering plant is a key model species owing to its comparatively simple genome and rapid development. Several groups have achieved targeted disruptions of endogenous genes at frequencies of a few per cent.

Rats and mice
These rodents are the most popular mammalian model organisms and ZFN technology has been applied to both of them using direct injection of mRNAs into one-cell embryos. The application of ZFNs to rats is particularly exciting. Although their larger size and physiological similarity to humans once made rats the preferred model, more powerful genetic tools for mice have led to the latter's ascendance among researchers. The success of ZFN mutagenesis in the rat heralds a comeback for the use of this organism in studies of human health and disease.



Agriculture
ZFNs have been used for gene disruption and gene replacement in cultures of maize and tobacco. Both species can be regenerated from such cultures, which facilitates delivery of ZFNs and characterization of sequence changes. The limitation in applying this approach to other species will be methods for delivery. Plants with directed changes in endogenous genes might be more publicly acceptable than the current genetically modified crops.



ZFN applications to animals of agricultural importance are also easily envisioned: for example, cows with altered milk proteins; pigs with organs manipulated for transplant to humans with reduced likelihood of rejection; and animals with improved growth characteristics.

Human gene therapy
The ability to manipulate DNA sequences with ZFNs has obvious implications for intervention in human genetic diseases. A clinical trial is currently in progress that involves disruption of the gene for the HIV co-receptor CCR5 with ZFNs. Loss of CCR5 has been shown to protect against propagation of HIV and progression to AIDS.

Current protocols rely on ZFN treatment of haematopoietic precursor cells in culture and transplantation back to the patient. This approach could be applied to other types of stem cells, as it has recently been shown that ZFNs increase gene targeting in ES and iPS cells. In the future ZFNs could be used, for example, to correct mutated genes in neurodegenerative diseases. A key concern is the possible production of unwanted off-target effects. Although this can be tolerated in model organisms, it could have serious, unintended consequences in humans. Nonetheless, progress in ZFN-induced gene targeting has been rapid and the prospects for the future are bright.

Abbreviations

CCR5, C-C chemokine receptor type 5; DBD, DNA-binding domain; DSB, double-strand break; ES, embryonic stem; HR, homologous recombination; iPS, induced pluripotent stem; ND, nuclease domain; NHEJ, non-homologous end joining; ZFN, zinc finger nuclease.

References

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Acknowledgements

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