

A technology for specifically correcting 'faulty' bases or mutations within genes of humans has been the 'Holy Grail' in genetic medicine. Gene targeting using zinc finger nucleases (ZFNs) - proteins custom-designed to cut at specific DNA sequences - appears to make this possible. These "artificial" proteins combine the non-specific endonuclease activity of FokI restriction enzyme with the ability of zinc-finger (ZF) motifs to specifically recognize a DNA triplet sequence. Amino acids involved in DNA recognition within the α -helix of the ZF motifs can be changed while maintaining the remaining amino acids as a consensus backbone to generate ZF motifs with new triplet sequence specificities. Normally, three such ZF motifs are linked together in tandem to generate a ZF protein (ZFP) that binds to a 9-bp site, which is a composite of the individual DNA triplet sub-sites recognized by each of the three ZF motifs. Binding of two three-finger ZFN monomers each recognizing a 9-bp inverted site is necessary because dimerization of the Fok I cleavage domain is required to produce a DSB. Therefore, three-finger ZFNs effectively have an 18-bp recognition site, which is long enough to specify a unique genomic address in plants and mammals.

The modular structure of zinc finger domain and modular recognition by zinc finger proteins make them the most versatile of DNA recognition motifs for designing such artificial DNA binding proteins. Each zinc finger consists of about 30 amino acids and folds into $\beta\alpha\beta$ -structures, which is stabilized by the chelation of a zinc ion by the conserved Cys²His² residues. Each finger typically recognizes a 3 bp DNA sequence by inserting the α -helix into the major groove of DNA. Binding of longer DNA sequences is achieved by linking several of these zinc finger motifs in tandem. The engineered ZFPs thus provide us with a powerful platform technology since other functionalities like nonspecific FokI cleavage domain (N), transcription activator domains (A), transcription repressor domains (R) and methylases (M) can be fused to a ZFPs to form respectively zinc finger nucleases (ZFN), zinc finger transcription activators (ZFA), zinc finger transcription repressors (ZFR) and zinc finger methylases (ZFM). Recent reports suggest that these engineered proteins find and cleave their chromosomal targets in cells; as expected, stimulate local homologous recombination to repair the DSB with the exogenously provided donor DNA. ZFNs thus offer a general mechanism by which to introduce a site-specific DSB within any genome. Computational informatics could play an important role in predicting ZFP specificity, identifying potential target sites and for designing the amino acid sequence of ZFP predicted to bind to a certain target site.

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