Meganucleases

Meganucleases are endonucleases that recognize specific dsDNA sequences with more than 12 bp. [1] The action of these is always based on the site-specific cleavage of the DNA by means of a nuclease and the triggering of the cell's gene repair mechanisms, through non-homologous end-joining (NHEJ) or homologous recombination (HR).

There are three types of meganucleases: ZFNs (zinc-finger nucleases), TALENs (transcription activator-like effectors nucleases) and CRISPR/Cas9.

Zinc finger nucleases are artificial restriction enzymes created by the fusion of a zinc finger DNA binding domain (constituted by zinc finger proteins and eukaryotic transcription factors) with a DNA cleavage domain (usually Fok1 restriction enzyme) that target specific sequences.

A pair of ZFN nucleases bind to the double stranded DNA and introduce a double strand break (the DNA binding domain recognizes a specific sequence and binds to it and the DNA cleavage domain introduces a DSB).

If donor DNA is present, the DSB is repaired inserting the donor DNA by homologous recombination; if there is no donor DNA, the break is repaired by non-homologous end joining, inducing targeted mutagenesis. [2]

Zinc-finger nucleases are used to manipulate the genomes of animals and plants. They are also used to create new isogenic disease models (genetic disease models).

ZFNs have also been used in a clinical trial of CD4+ human T cells with the CCR5 gene disrupted by zinc-finger nucleases to be safe as a potential treatment for HIV/AIDS.

Other applications include: functional genomics/target validation, creation of gene knockouts in multiple cell lines, complete knockout of genes not amenable to RNAi, cell-based screening, creation of knock-in cell lines with promoters, fusion tags or reporters integrated into endogenous genes, cell line optimization, creation of cell lines that produce higher yields of proteins or antibodies.

Although TALENs are similar to ZFNs in the way they work, TALENs have a greater flexibility in selecting target sequences than that of ZFNs. [3] TALENs have a Fok1 restriction endonuclease as DNA-cleavage domain, that is fused to a TALE central repeat domain that consists of identical repeating of 33–35 amino acids, that vary on positions 12 and 13. These endonucleases can recognize only 1 nucleotide. [4]

The scientific community has been using these proteins to induce highly efficient gene targeting in mammalian cells. But the recent development of artificial meganucleases opened up a range of new opportunities. Their ability to cleave chosen sequences can possibly be used in therapy to introduce transgenes in a determined loci, to correct mutated genes or to inactivate others. However, it can take a while until meganucleases can be used in the medical field. There is a good appreciation of the efficiency of these proteins on models in cultured cells but not on primary cells or differentiated tissues, where each case can be different from another. As so, they primarily have applications in the field of agribusiness, protein production and others. [5]

I-SceI, natural pioneer meganuclease, is the prototypical meganuclease used for genome engineering. [6]

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