JUST AGRICULTURE
multidisciplinary e-Newsletter

Gene Editing: A Tool for Genetic Makeup

Dr. Gunnjeet kaur
Assistant Professor, School of Agricultural Sciences, Dr. K. N Modi University Newai, Rajasthan

ARTICLE ID: 74

Introduction

People have long sought and used scientific knowledge to improve the conditions of human life. From breeding crops and the domestication of livestock to modern health care, the biological sciences underpin the possibility of human beings exercising ever greater levels of control over the biosphere, including their environment, the other living beings with which they share it and their own bodies. Contemporary molecular biology affords a particularly powerful set of tools that form the basis of a range of technologies in fields as diverse as medicine, agriculture, industrial production, and environmental management. What we will refer to as 'genome editing' is the practice of making targeted interventions at the molecular level of DNA or RNA function, deliberately to alter the structural or functional characteristics of biological entities. These entities include complex living organisms, such as humans and animals, tissues and cells in culture, and plants, bacteria and viruses. Characteristics of many kinds, from the colour or number of blooms in flowering plants, to some disease traits in animals and plants, can be altered, though the extent to which, and ease with which, such alterations can be made is highly variable.

Gene Editing

Gene-editing technologies use proteins, called enzymes, to cut targeted areas of DNA within a genome. Cells repair these cuts but if no instructions are provided for the repair, the repair process can make mistakes, resulting in altered DNA sequences. If specific DNA repair information is provided, however, the cell will use this to repair the cut in the way it is instructed. The use of this process provides an opportunity for researchers to modify the genome, by providing slightly different repair information from what was there before.

What are the new gene-editing technologies?

The three main new gene-editing technologies which have been developed to do this are ZFNs and TALENs and CRISPRs

JUST AGRICULTURE
multidisciplinary e-Newsletter

 # ZFNs (Zinc-finger nucleases) used as a bacterial DNA cutting enzyme that has been combined with proteins called 'zinc fingers', which can be customized to recognize a specific section of DNA . In 2005, this technology was first used to edit DNA in human cells.

Figure 1. Each Zinc Finger Nuclease (ZFN) consists of two functional domains: a.) A DNA-binding domain comprised of a chain of two-finger modules, each recognizing a unique hexamer (6 bp) sequence of DNA. Two-finger modules are stitched together to form a Zinc Finger Protein, each with specificity of ≥ 24 bp. b.) A DNA-cleaving domain comprised of the nuclease domain of Fok I. When the DNA-binding and DNA-cleaving domains are fused together, a highly-specific pair of 'genomic scissors' is created.

Target Applications

- **In Functional Genomics/Target Validation:** Creation of gene knockouts in multiple cell lines and complete knockout of genes not amenable to RNAi
- **In Cell-based screening**: Creation of knock-in cell lines with promoters, fusion tags or reporters integrated into endogenous genes
- **In Cell Line Optimization**:Creation of cell lines that produce higher yields of proteins or antibodies

 # TALENs (Transcription activator-like effector nucleases) again use a DNA-cutting enzyme combined with proteins from bacteria that target areas of DNA, in a similar way to the zinc finger proteins. TALENs can be designed with long DNA recognition sections, and therefore

JUST AGRICULTURE
multidisciplinary e-Newsletter

tend to have lower unintended off-target cut sites, which can occur when parts of a genome have an identical or near-identical sequence to the target site.

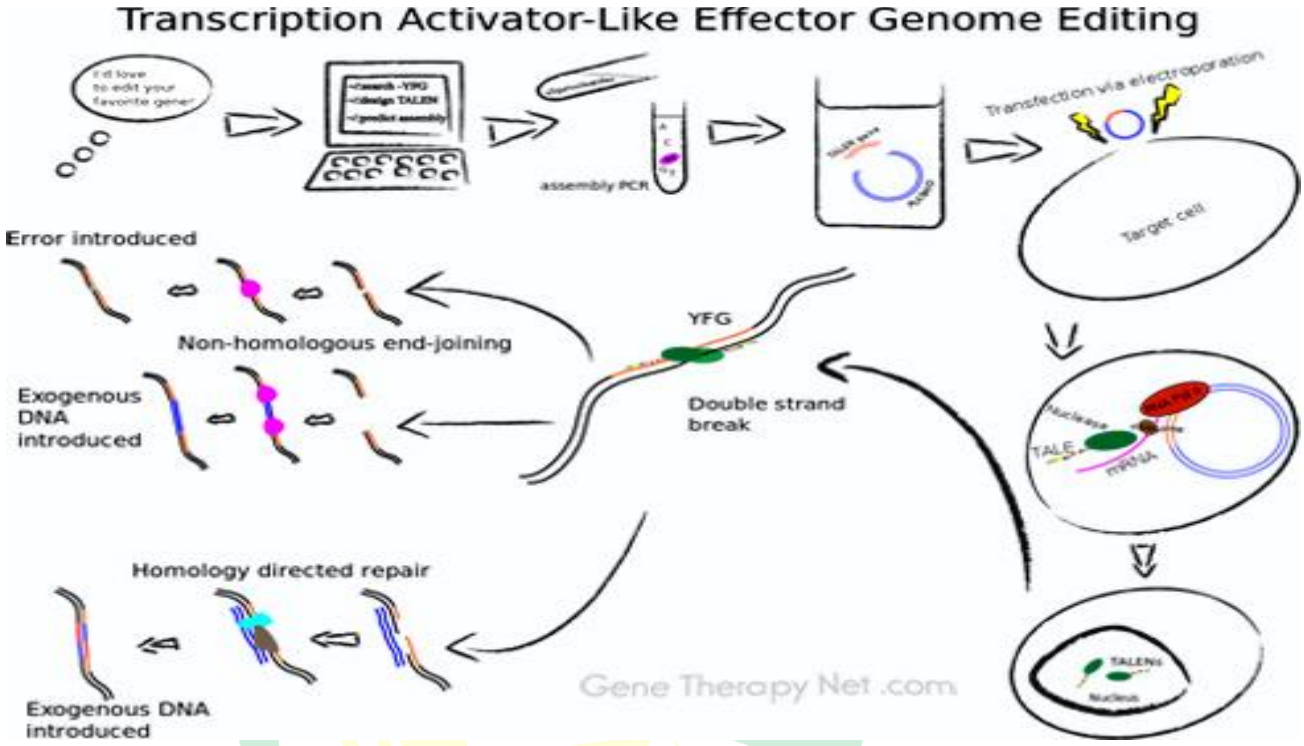

Figure 2. Workflow of genome editing of Your Favorite Gene (YFG) using TALEN technology. The targent sequence is identified, a corresponding TALEN sequence is engineered and inserted into a plasmid. The plasmid is inserted into the target cell where it is translated to produce the functional TALEN, which enters the nucleus and binds and cleaves the target sequence.

Target Applications

- Edit genomes by inducing double-strand breaks (DSB) for repair mechanisms.
- Non-homologous end joining (NHEJ) reconnects DNA.
- Chromosomal rearrangement .
- DNA can be introduced into a genome through NHEJ in the presence of exogenous double-stranded DNA fragments.
- Homology directed repair can also introduce foreign DNA at the DSB as the transfected double-stranded sequences are used as templates for the repair enzymes.

JUST AGRICULTURE
multidisciplinary e-Newsletter

 # CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), Bacteria possess an immune system which recognises invading viral DNA and cuts it up, making the invading virus DNA inactive. It is type of immune system .CRISPR, unlike ZFNs and TALENs allows for many DNA sites to be edited simultaneously and easily . It is also the most affordable and programmable genome editing technology. While much more accurate than earlier genetic modification technologies, there can still be unintended off-target effects, although these are detectable and new research is rapidly improving the technology's accuracy.

Target Applications

- Clustered regulatory interspaced short palindromic repeats (CRISPR) associated 9 (Cas9) bacterial endonuclease and a short guide RNA (gRNA) to target the Cas9 protein to a genomic region of interest.
- The target efficiency is 50% in human cells.
- The Cas9 system has been reported to have efficiencies up to >70% in zebrafish and plants
- 2–5% in induced pluripotent stem cells.
- A widely used method to identify mutations is the T7 Endonuclease I mutation detection assay .This assay detects heteroduplex DNA that results from the annealing of a DNA strand, including desired mutations, with a wildtype DNA strand .

Conclusion:

The rapid development and improvement of genome-editing tools provide investigators with three well-characterized options for experiments as diverse as forward genetic screens to correction of pathogenic mutations in iPSC-derived human cells. ZFNs, TALENs, and CRISPRs can all generate site-specific DSBs with varying degrees of specificity and efficiency. The early uses of these systems have demonstrated remarkable new possibilities and allowed for the creation of model systems in a wide variety of organisms. With each iteration, the technology has improved, and the prospects for the study and treatment of human disease with genome editing have never been better.