AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 01, Issue: 05 (December, 2024)
Available online at http://www.agrimagazine.in
©Agri Magazine, ISSN: 3048-8656

Beyond The Helix: New Frontiers in Genome Editing

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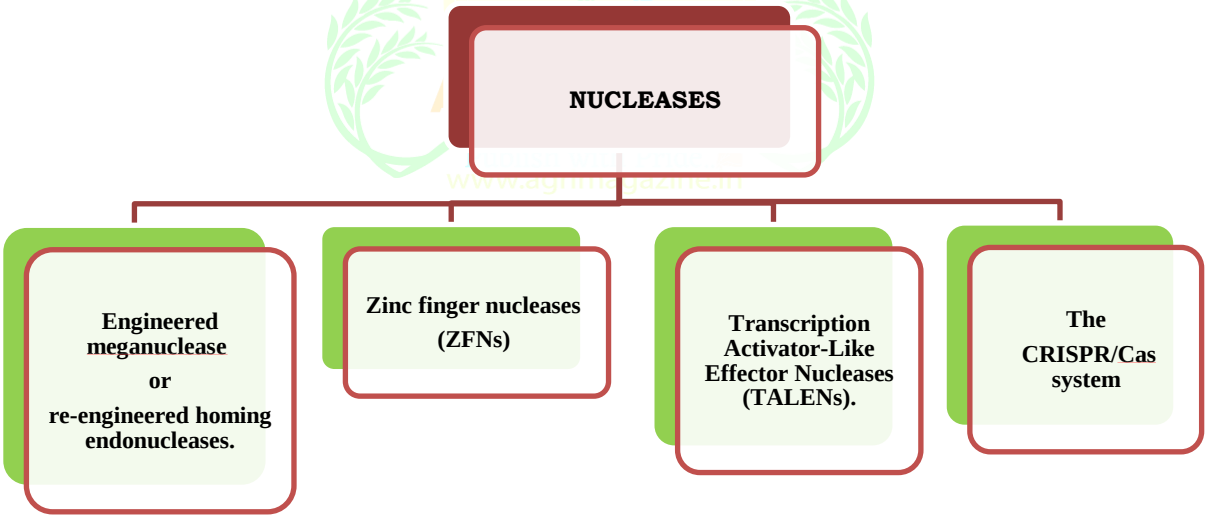
What is genome editing?

- Genome editing, also known as genome editing with engineered nucleases (GEEN), is a form of genetic engineering in which DNA is inserted, altered, or removed from a genome using chemically made nucleases, or "molecular scissors.
- Nucleases generate specific double-stranded breaks (DSBs) in the genome and use the cell's own mechanisms to repair the caused break through natural processes such as homologous recombination (HR) and non-homologous end-joining (NHEJ).
- Genome editing was selected by Nature Methods as the Method of the Year in 2011. Bak *et al*., (2018)

Key Features of Genome Editing

- **Precision**: Genome editing tools are designed to target specific sequences of DNA with minimal unintended changes, making them highly accurate.
- **Versatility**: These techniques can be applied to a wide range of organisms, including humans, animals, plants, and microorganisms.
- **Efficiency**: Genome editing can quickly introduce genetic changes, often in just one or a few generations.

Types of nucleases used



1. Zinc Finger Nucleases (ZFNs)

- **Zinc Finger Nucleases** (ZFNs) are a type of genome editing tool that allows for the targeted1modification of DNA within living organisms. They are a powerful technology used to introduce double-strand breaks at specific locations in the genome, which can then be repaired by the cell's natural DNA repair mechanisms. ZFNs are one of the earlier

genome editing technologies, and although newer tools like CRISPR have become more popular due to their simplicity, ZFNs have been important in advancing genetic engineering.

Key Components of Zinc Finger Nucleases:

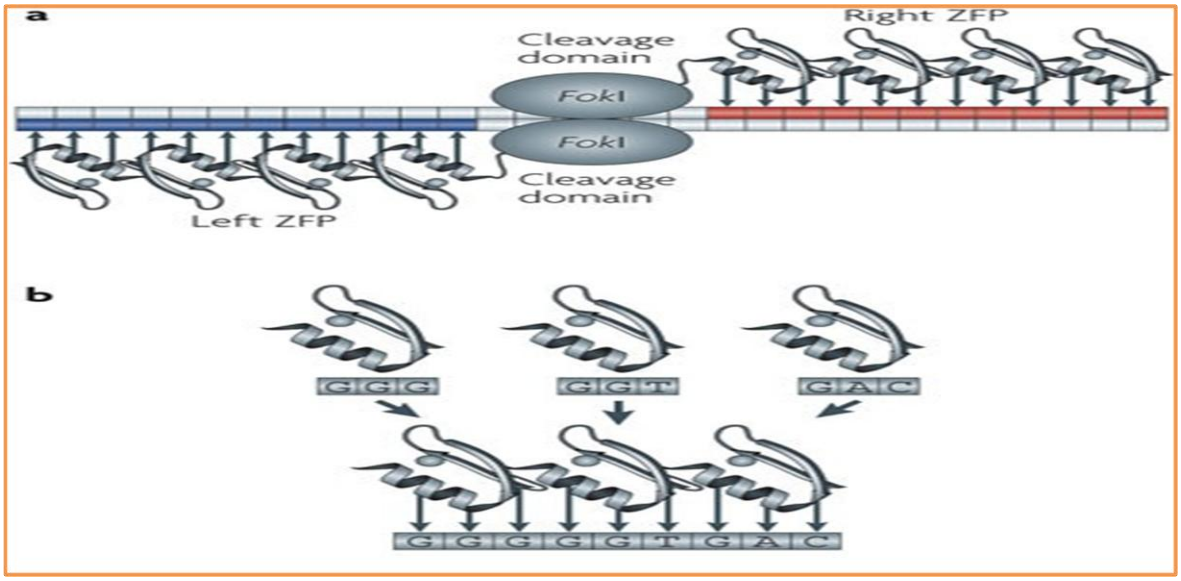
1. **Zinc Finger Proteins**: These are small, naturally occurring protein motifs that can recognize specific sequences of DNA. Zinc fingers are coordinated by zinc ions, and they bind to particular sequences in the DNA by recognizing 3 to 4 base pairs at a time. By engineering different zinc finger proteins, it is possible to target different sequences of DNA.
2. **Nuclease Domain (FokI)**: The second essential component of ZFNs is a nuclease domain, often derived from the **FokI restriction enzyme**. This enzyme cuts DNA at a specific site. However, FokI only cuts DNA when two ZFN molecules, each recognizing half of the target site, bind to their complementary regions in the genome, allowing for a double-strand break. This unique feature ensures that ZFNs can only cut when both zinc fingers are correctly positioned on the DNA.

How Zinc Finger Nucleases Work:

1. **Binding to Target DNA**: The engineered zinc finger proteins are designed to bind to a specific DNA sequence in the genome. By combining several zinc fingers, each targeting a different sequence of DNA, ZFNs can be programmed to bind to longer, unique sequences. This specificity allows for precise targeting of genes in an organism's genome.
2. **Creating Double-Strand Breaks**: Once the ZFNs bind to their target site, the FokI nuclease domains dimerize (pair together) and create a double-strand break in the DNA. This break can be used to induce changes in the DNA sequence in two main ways:

- **Non-homologous end joining (NHEJ)**: This is a DNA repair mechanism that often leads to small insertions or deletions (indels) at the break site, potentially disrupting the target gene.
- **Homology-directed repair (HDR)**: If a repair template is provided, the cell can use the template to insert or replace DNA sequences at the break site, allowing for precise edits, such as correcting mutations or inserting new genes.

3. **Editing the Genome**: After the DNA is cut, the cell's repair machinery takes over. The cell might either repair the break by creating a mutation (NHEJ) or by copying the desired sequence from an external DNA template (HDR), leading to the desired genetic change.



2. Transcription Activator-Like Effectors (TALEs) are a family of proteins originally discovered in *Xanthomonas* bacteria, which are plant pathogens. These proteins play a crucial role in the ability of these bacteria to infect plants by regulating the expression of plant genes. TALEs are unique in their ability to specifically bind to DNA sequences in the host's

genome, thus activating or repressing the expression of certain genes. Priyadarshan P. M. (2017)

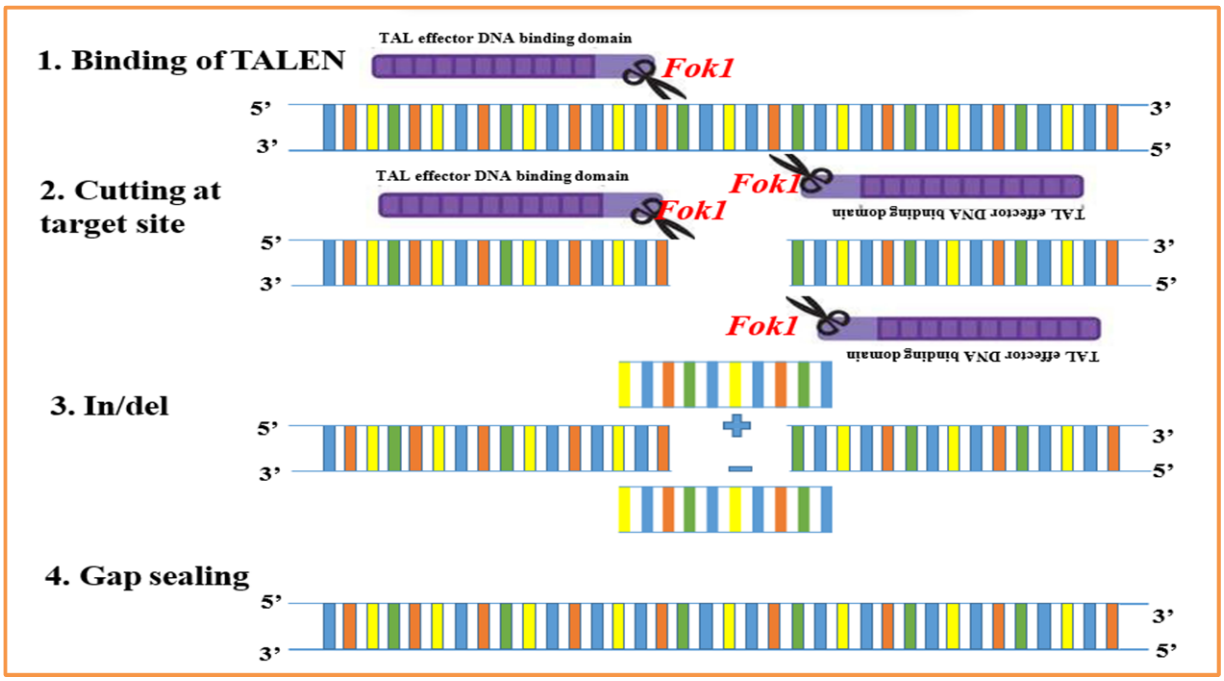
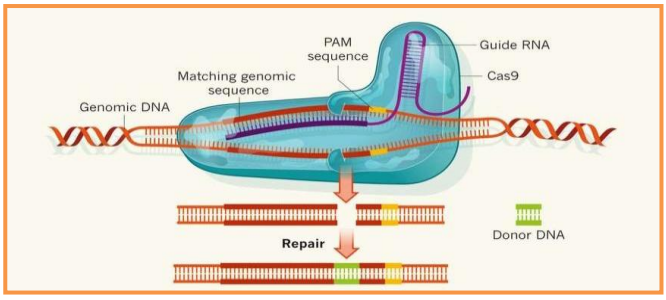


Fig. Mechanism of Transcription activator-like effector nucleases (TALENS)

3. CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats):

The CRISPRs (Clustered **Regularly** Interspaced Short Palindromic Repeats) and CRISPR-associated proteins (Cas) form an RNA-guided adaptive immune system that protects bacteria and archaea from phage infection.

CRISPR arrays were first identified in the E. coli genome (Ishino *et al*., 1987).



Components of CRISPR

1. Protospacer adjacent motif (PAM)
2. CRISPR-RNA (crRNA)
3. trans-activating crRNA (tracr RNA)
4. Cas proteins

- Cas9 protein is guided by a structure formed by a CRISPR RNA (crRNA), which contains a segment determining target specificity and a trans-activating CRISPR RNA (tracrRNA), which stabilizes the structure and activates Cas9 to cleave the target DNA. The presence of a protospacer-adjacent motif (PAM) (NGG or NAG) sequence directly downstream from the target DNA, is a prerequisite for DNA cleavage by Cas9.

4. Engineered homing endonucleases: Also known as mega nucleases.

- First tool used for double strand break-induced genome manipulation.It is Occur naturally in Yeast and in *Chlamydomonas*.The very first meganuclease, *I - SecI* used for intron homing (insertion of the intron into the target site).
- In these enzymes binding site and restriction site occur within same unit hence difficult to modify. Abdallah *et al*., (2015)

Conclusion on Genome Editing Techniques

- Genome editing techniques have revolutionized the field of molecular biology and genetic engineering, offering unprecedented precision in modifying the genetic material of living organisms. Technologies like **CRISPR-Cas9**, **TALENs**, and **Zinc Finger Nucleases** (ZFNs) have each contributed uniquely to our ability to target specific genes, introduce mutations, or correct genetic defects.

1. **CRISPR-Cas9** is perhaps the most widely recognized and utilized technique due to its simplicity, efficiency, and versatility. It has democratized genome editing, enabling researchers to edit the genomes of a wide variety of organisms with relative ease.
2. **TALENs** and **ZFNs**, while more complex and less commonly used than CRISPR, offer higher specificity in certain applications and may be preferable when off-target effects or precision are critical. These techniques have also found particular success in plant and animal breeding, as well as in therapeutic applications.

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