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An Overview of Genome Editing Tools

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SUMMARY

The emergence of genome manipulation methods promises a real revolution in biotechnology and genetic engineering. Genome editing of model organism is essential for gene function analysis and is thus critical for human health and agricultural production. This system harnesses the DNA Repair system of the cell to create modification in the target DNA sequence.

INTRODUCTION

Genome-wide editing is not a new field, and in fact, research in this field has been active since the 1970s. (Ishino *et al*. 1987, Nakata *et al*., 1989) The real history of this technology started with pioneers in genome engineering. Genome editing and gene editing are techniques that incorporate site-specific modifications into genomic DNA using DNA repair mechanisms. Gene editing differs from genome editing by dealing with only one gene. Targeted editing of the genomes of living organisms not only permits investigations into the understanding of the fundamental basis of biological systems but also allows addressing a wide range of goals towards improving productivity and quality of crops. This includes the creation of plants with valuable compositional properties and with traits that confer resistance to various biotic and abiotic stresses. During the past few years, several novel genome editing systems have been developed; these include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspaced short palindromic repeats/Cas9 (CRISPR/Cas9), These highly versatile genome-editing technologies has provided investigators with the ability to rapidly and economically introduce sequence-specific modifications into the genomes of a broad spectrum of cell types and organisms.

Genome Editing Tools

ZFNS (Zinc-Finger Nucleases):-

It is the first generation or most commen types of genome editing tools found in eukaryotes that use chimerically engineered nucleases which were developed after the discovery of the working principles of the functional Cys2-His2 zinc finger (ZF) domain .It consists of approximately 30 amino acid residues in a conserved $\beta\beta\alpha$ configuration. (Cathomen T. and Keith Joung J.,2008, Petolino J. F. 2015) It is fusions of the non-specific DNA cleavage domain from the Fold restriction endonuclease with zinc-finger proteins. ZFN dimers induce targeted DNA double-strand breaks (DSBs) that stimulate DNA damage response pathways. The binding specificity of the designed zinc-finger domain directs the ZFN to a specific genomic site. Crystallographic structure analysis showed that the Cys2-His2 ZF proteins bind to DNA byinserting an α-helix of the protein into the major groove of the DNA-double helix. Each ZF protein has the ability to recognize 3 tandem nucleotides in the DNA. ZFNs provide advantages over other tools with respect to efficiency, high specificity, and minimal nontarget effects and current efforts are focused on further improving design and delivery as well as expanding their applications in diverse crops of interest.

TALENs (Transcription Activator-Like Effector)

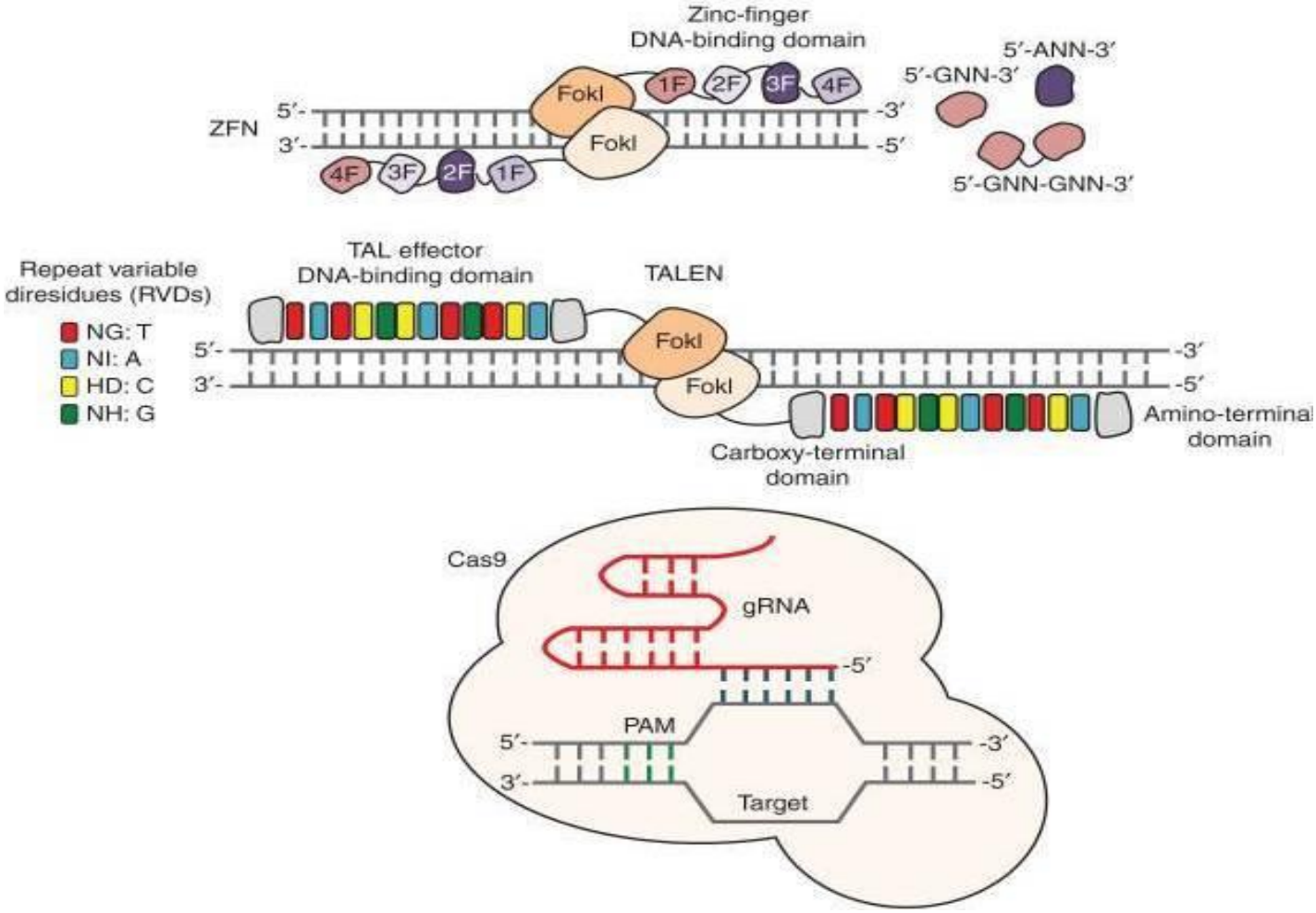
It is cheaper, safer, more efficient, and capable of targeting a specified region in the genome nucleases are fusions of the FolA cleavage domain and DNA-binding domains derived from TALE proteins. TALEs contain multiple 33-35 amino acid repeat domains that each recognizes a single base pair. Like ZFNs, TALENs induce targeted DSBs that activate DNA damage response pathways and enable custom alterations.

CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats)

The CRISPR/Cas system is the most recent platform in the field of genome editing. The system was developed in 2013 and is known as the third generation genomic editing tools. CRISPR are loci that contain

multiple short direct repeats, and provide acquired immunity to bacteria and archaea. The key element of this system are Cas protein , Cas9 endonuclease with its different versions, can cleave at a specific target site with the help of two short RNA molecules namely CRISPR RNA (crRNA) and trans-encoded CRISPER RNA (tracrRNA) for sequence-specific silencing of invading foreign DNA. These two RNA molecules form a chimeric RNA molecules named as single guide RNA (sg RNA) . This RNA along with Cas 9 protein able to from an RNA–guided endonuclease that regulate sequence-specific cleavage in the genome.

Diagrammatic Illustratration of the Mechanisms of Genome Editing Tools



From *Top* to *Bottom*:

Zinc-finger nucleases (ZFNs), transcription activator-like effector (TALE) nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9).

ZFNs recognize target sites that consist of two zinc-finger binding sites that flank a 5- to 7-base pair (bp) spacer sequence recognized by the FokI cleavage domain.

TALENs recognize target sites that consist of two TALE DNA-binding sites that flank a 12- to 20-bp spacer sequence recognized by the FokI cleavage domain.

The Cas9 nuclease is targeted to DNA sequences complementary to the targeting sequence within the single guide RNA (gRNA) located immediately upstream of a compatible protospacer adjacent motif (PAM). DNA and protein are not drawn to scale.

CONCLUSION

Genome editing tools are becoming popular molecular tools of choice for functional genomics as well as crop improvement. Many examples exist currently where these editing systems are being harnessed for unprecedented understanding of plant biology and crop yield improvement through rapid and targeted

mutagenesis and associated breeding, Because of their several attractive features such as simplicity, efficiency, high specificity, and amenability to multiplexing, genome editing technologies described here are revolutionizing the way crop breeding is done and paving the way for the next generation breeding.

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