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Genome Editing: A Novel Tool for Disease Resistance

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Abstract

The recent advent of genome-editing technologies has enabled a new paradigm in which the sequence of the human genome can be precisely manipulated to achieve a therapeutic effect. This includes the correction of mutations that cause disease, the addition of therapeutic genes to specific sites in the genome, and the removal of deleterious genes or genome sequences. This article presents the mechanisms of different genome-editing strategies and describes each of the common nuclease-based platforms, which includes CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats which are associated with protein 9), TALEN (transcription activator-like effectors nuclease), and ZFN (zinc finger nuclease). These technologies are beginning to be used for new approaches in a variety of areas including research, medicine, agriculture, biotechnology and have the potential to be used in pest control. These new technologies pose challenges for regulators who will find it harder to distinguish between genetic changes in organisms generated by conventional breeding, gene editing, or natural mutation.

Keywords: CRISPER/Cas 9, TALEN, ZFN

Introduction

Genome editing is a technique that can be used to make changes to a cell's DNA (the term 'genome' simply refers to all of the DNA in a cell). DNA sequences contain four different components of nitrogenous bases are represented by the letters A, C, G and T. Making changes to a cell's DNA has the potential to affect how that cell functions. Genome editing is used to make precise genetic modifications for various purposes, such as studying the gene function, biological mechanisms, pathology of diseases and to correct mutations in genes that cause one of the 10,000 disorders that result from mutations in a single gene (monogenic disorders) and to prevent such disorders from being inherited.

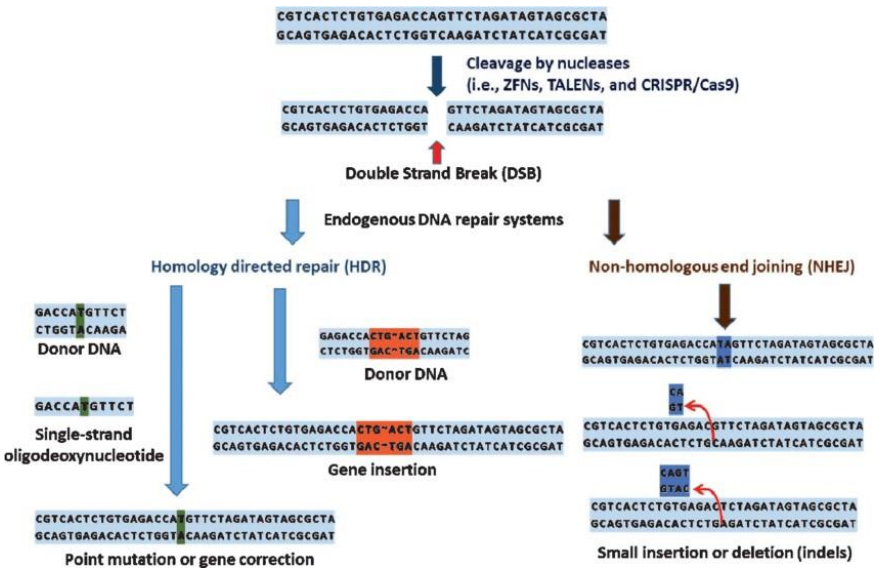
The intended changes include target gene mutagenesis, insertion and replacement, suppression or activation of the gene expression, and target chromosomal rearrangement. Gene-editing technologies use proteins, called enzymes, to cut targeted areas of DNA within a genome. Cells repair these cuts but if 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. In this way, it is possible to use gene editing to change a version of a gene from one that causes disease to one that does not (for example gene variants that contribute to Parkinson's disease (Soldner et al., 2016) or genetic metabolic disorders (Hao et al., 2014), or choose the version of a gene that confers better resistance to disease in agricultural plants and animals. It is also possible to use the technique to modify genes without introducing foreign DNA sequences. For example, gene editing can be used to switch off genes (Maeder and Gersbach, 2016) in laboratory-grown cells to identify their function (Shalem et al., 2014), or to switch off genes that are causing disease, such as in animal models of Huntington's disease.While technologies to make cuts in DNA have been known since the 1970s, using them in a controlled and accurate way, and in organisms whose genome is poorly understood, has been a major hurdle. However, in the last 10 years, researchers have identified, or created, proteins that permit gene-editing technologies to make gene edits in specific areas of DNA, rather than introducing these changes randomly into the genome.

Three main classes of endonucleases, including zinc finger nucleases (ZFNs) , transcription activator-like effector nucleases (TALENs) , and CRISPR/Cas9 system are known as the main tools in genome editing.

Genome-Editing Techniques

The diverse array of genetic outcomes made possible by these technologies is the result, in large part, of their ability to efficiently induce targeted DNA double-strand breaks (DSBs). These DNA breaks then drive activation of cellular DNA repair pathways and facilitate the introduction of site-specific genomic modifications. This process is most often used to achieve gene knockout via random base insertions and/or deletions that can be introduced by nonhomologous end joining (NHEJ) (Fig. 1) (Bibikova et al., 2002). Alternatively, in the presence of a donor template with homology to the targeted chromosomal site, gene integration, or base correction via homology-directed repair (HDR) can occur (HDR) (Fig. 1). Indeed, the broad versatility of these genome-modifying enzymes is evidenced by the



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fact that they also serve as the foundation for artificial transcription factors, a class of tools capable of modulating the expression of nearly any gene within a genome. Representative genome-editing techniques are discussed below (Figure 1).

Zinc-finger nucleases (ZFNs)

ZFNs use a bacterial DNA cutting enzyme (Kim et al., 1996) that has been combined with proteins called 'zinc fingers', which can be customized to recognise a specific section of DNA. ZFNs are small (one-third of the size of TALENs and much smaller than CRISPR) so they are easier to package inside delivery vehicles, such as viruses, to enable them to reach their targets in cells for genome editing-based therapies (Chen and Goncalves, 2016).

TALENs

TALENs (transcription activator-like effector nucleases) again use a DNA-cutting enzyme combined with proteins from bacteria [Joung and Sander., 2013] 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 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.

CRISPR

Bacteria possess an immune system which recognises invading viral DNA and cuts it up, making the invading virus DNA inactive. This type of immune system is known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) [Marraffini., 2015].In 2016, researchers further improved on the performance of CRISPR by allowing for editing of single DNA letters.

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 [Kleinstiver et al., 2016].

For simplicity and consolidation, an overview of genome-editing techniques is presented in Figure 2.

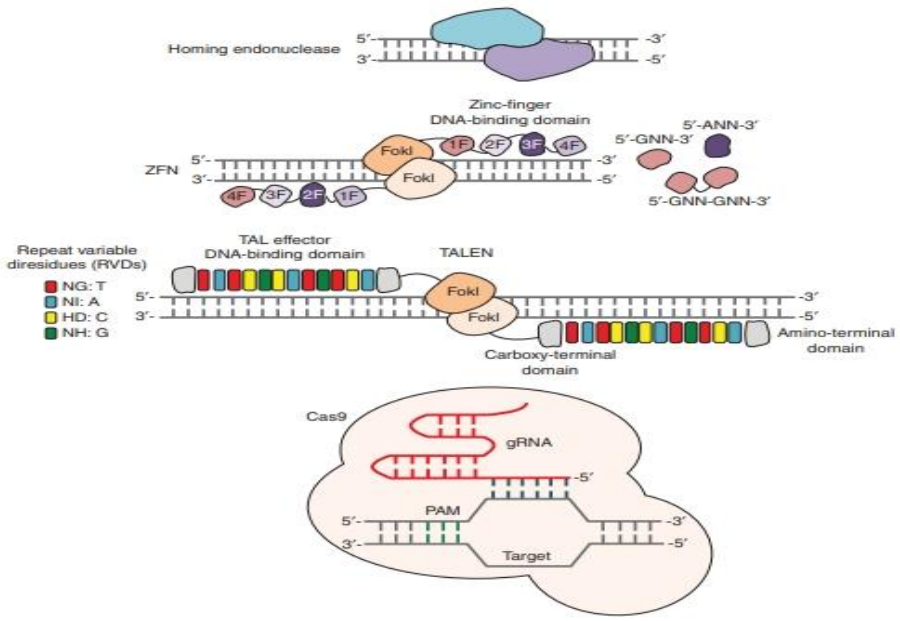

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Figure 2 Cartoons illustrating the mechanisms of targeted nucleases. From top to bottom: homing endonucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector (TALE) nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9). Homing endonucleases generally cleave their DNA substrates as dimers, and do not have distinct binding and cleavage domains. 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

It can be concluded that the technologies of gene editing provide a great platform for researchers to develop a therapeutic agent to treat the disease that is mainly generated through genetic disorders. With the help of such innovative technologies, researchers can develop new genomes that can be used in the treatment of different kinds of diseases especially cancer. The process is executed by cutting the DNA sequence with specific enzymes and thus this phenomenon is referred to as nuclei engineering and through this process, the DNA can be modified and can be further inserted into the gene of interest.

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