

Genome Editing: Innovation in Molecular Biology

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Abstract

Deletion, insertion, or substitution of DNA at a precise location in the genome of an organism is termed as Genome editing. It is usually accomplished *in vitro* using genetically engineered restriction enzymes endonucleases also called as *molecular scissors*. There is a numerous way to edit genome using different genome editing systems including CRISPR-Cas9, ZFNs or TALENs. Each of these systems possesses unique properties that are exploited for an organism benefits.

Keywords: Nucleases; CRISPR-Cas9; ZFNs

Introduction

Genome editing is a novel approach that is gaining momentum by making precise and unique edits in the existing genetic code of a living organism. This innovating molecular technique enables addition, deletion and substitution of bases by incorporating detectable changes in the DNA of an organism [1]. In comparison with classical genetic engineering which is the cleavage and random insertion of a foreign gene or DNA sequence from a different specie to another. Genome editing technology holds a different concept. It edits the DNA or genes at much defined localities in the genome [2].

Genome Editing Working Principle

The core concept behind genome editing is the use of restriction nucleases that make explicit cuts in the target DNA by recognition of specific restriction sites also called as recognition sites within the target sequence or gene. The cleavage location is then repaired by DNA repair system of the cell. These repairs can be tricked to add, remove, or substitute a series of letters in the genetic code [3]. Thus enables the introduction of known desired alleles in the target organism. Breaks in the double stranded DNA produced at exact position in the DNA by restriction endonucleases are mended by DNA repair pathway of the cell without template DNA. Outcome of this modification is a change in a single nucleotide either by insertion or deletion at the DNA cut site. Though the position of the cleaved site is precise but the exact alteration that occurs when the DNA is repaired is random. So there might be a number of different possibilities producing minor sequence changes [3].

Types of genome editing

Small DNA changes: Engineered endo nuclease designed to cleave at specific localities in the DNA. DNA segment with the engineered nuclease after cleavage will be recognized by the cells natural repair system that will ligase the two cleaved ends together. This repair mechanism is not 100% efficient resulting in the loss or gain of few nucleotide bases during the repair process. A small change in the nucleotide sequence will result in the alteration of the function of that segment of DNA. As a result a gene may either become nonfunctional or doesn't function properly [4,5].

Removal of a section of DNA: Endonucleases are designed to make cuts on either side of the DNA to remove a segment. After cleavage, cells normal DNA repair mechanism will identify the damage and initiate the repair process but may mistakenly join the wrong ends of DNA together, removing the DNA in between the two cuts [6].

Insertion of section of DNA: Genome editing could be used to direct the cells natural DNA repair system to insert a segment of

DNA into a genome. Before cell division, cell copies all of its DNA so a complete copy of the genome is transferred to the two resultant daughter cells. Since DNA repair is a homology directed repair process [7]. Designed endonucleases cleave at precise location in the DNA. After cleavage, a DNA segment homologous in sequence to the site of the cleavage is introduced [8]. The break is repaired by using this modified DNA segment as template. As a result a new DNA segment is inserted or an existing segment of DNA is substituted by an altered one [9].

Genome editing systems

There are numerous types of engineered restriction nuclease systems used in genome editing. They all contain a nuclease part that cleaves the DNA and a DNA-targeting part to identify the DNA sequence that has been cleaved.

CRISPR-Cas9: CRISPR-Cas9 is the mostly used cost effective genome editing system. These clustered regularly interspaced short palindromic repeats targets DNA. It comprised of a guide RNA molecule that binds to specific DNA bases through complementary base pairing. Cas9 is CRISPR-associated protein 9, nuclease part that cleaves the DNA. This system was originally reported in bacteria that uses it to destroy invading viruses [9,10].

ZFNs: Zinc-finger nucleases (ZFNs) are another type of nucleases in which zinc-finger proteins is the DNA-binding part of ZFNs which bind to about three DNA bases [11,12]. FokI is the nuclease part of ZFNs which cleaves the DNA [13]. Two FokI molecules collaborate together to make a cut in the target DNA molecule so it works in pairs.

TALENs: In transcription activator-like effector nucleases, DNA-binding domain is made of TALE (transcription activator-like effector domains) [14,15]. There are four different TALE domains for four different nucleotide bases, so they can be engineered to bind to precise DNA regions with much ease than ZFNs. FokI is the nuclease part of TALENs. Two FokI molecules must come together to make a cleavage in the DNA, so two TALENs are made, one for each strand [16,17].

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Received June 30 2017; Accepted July 10, 2017; Published July 20, 2017

Citation: Khan NT (2017) Genome Editing: Innovation in Molecular Biology. Hereditary Genet 6: 185. doi:10.4172/2161-1041.1000185

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Applications of genome editing

Genome editing has been used for a variety of purposes such as for the development of human disease models, understanding complex biological pathway etc., it has infinite range of promising applications.

Biomedical research studies: Genome editing is employed to alter the DNA in organisms to have an insight of the fundamental biological pathways and its underlying mechanism [18].

Diseases treatment: Genome editing has been used to cure different genetic diseases such as *in vitro* modification of human blood cells that are then introduced into the body to treat diseases like leukemia and AIDS [19]. Besides, genetic diseases including muscular dystrophy and hemophilia could also be treated [20,21].

Agricultural biotechnology: Genetic modification of food crops has been done through the use of genome editing technology to make them disease resistance as well to increase their yield. For example, CRISPR-Cas9 and TALENs editing systems have been used to create modification in the multiple alleles within hexaploid bread wheat to make them resistant against powdery mildew. And such genomic modification is heritable. It was reported that by using TALENs, genes in the fatty acid metabolic pathway in soybean were knocked out to create a modified plant with few metabolic components etc. [22,23].

Crops and livestock: Increased product yield, disease resistance potential, and abiotic tolerance, was achieved in different plants and animals by employing genome editing technology [24].

Industrial biotechnology: Production of biopharmaceuticals, biofuels, biodegradable plastics, biomaterials etc is the recent applications of genome editing.

Biomedicine: Nanoparticles mediated drug delivery systems, xenotransplantation, gene therapies, control and prevention of genetic diseases [25].

Reproduction: Preventing the inheritance of a disease trait [26].

Animal breeding: Genome editing is used to create genetically modified animals with improved phenotypes by modifying a less desirable allele rather than by means of conventional selective breeding. Therefore this cost effective method is more like precision breeding where breeders can introduce the specific sequences that they would like to select [27,28].

Targeting efficiency and of incidence of off-target mutations of different genome editing systems

Efficiency of genome targeting and the proportion of anticipated transformation achieved, is one of the most significant considerations to assess the importance of this tool. Targeting efficiency of CRISPR-Cas9 is more than ZFNs and TALENs [1]. It was reported that targeting efficiency of TALENs and ZFNs in human cells lie from 1% to 50% [29-31]. However an efficiency percentage of >70% was reported in zebrafish [32] and plants [33] while 2% to 5% was achieved in stem cells [34] using RISPERS Cas9. Improved germline transmission using dual sgRNAs could increase the efficiency of genome targeting up to 78%, for example in single celled embryo of mouse [35]. Frequency of off-target mutations is an important parameter that usually occurs at sites that have little nucleotide difference in comparison to the original sequence localized adjacent to PAM sequence. A single nucleotide change in the PAM sequence or a five nucleotide mismatches within the protospacer region can easily be tolerated by CRISPR Cas9. Detection of off-target mutations is not that easy but it requires sequencing of whole-genome to eliminate them. Recently CRISPR Cas9 editing system has been

modified to reduce the probability of off-target mutations by either two extra guanine nucleotides addition to the 5' end or by employing condensed gRNA (condensed within the crRNA-derived sequence). Another approach is the use of "paired nickases" that employed D10A Cas9 and two sgRNAs complementary to the adjacent area in the target site. Thus, only a single nick is produced in off-target locations and, therefore, results in insignificant off-target mutations [36].

Conclusion

Genome editing technology is an innovation in the field of molecular biology. Designer nucleases is responsible for its high efficiency and versatility enabling precise genomic modification in the target. Thus, genome editing holds a promising future in the field of genome engineering.

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