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CRISPR/Cas9 System and Gene Editing Technology

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ABSTRACT

This review on gene editing technologies summarizes the features, application areas and future of the CRISPR/Cas9 genome editing system. Recently, the aim of searchers has been to improve cost-effective and reliable ways to perform targeted changes to the genome of living cells. In particular, genome editing technologies involving DNA-binding proteins such as Zinc Finger Nuclease (ZFN) and Transcription Activator-Like Effector Nucleases (TALEN) have made great progress. Thanks to the CRISPR/Cas9 system, which has been developed in addition to sensitive and efficient systems to edit the genome in biological sciences, progress has been made in many fields such as molecular biology, biomedicine and medicine. Moreover, it has contributed to important developments in the field of genome engineering through targeted breaks in the DNA of almost every organism and cell type.

CRISPR/Cas9 Sistemi ve Gen Düzenleme Teknolojisi

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ÖZ

Gen düzenleme teknolojileri üzerine yapılan bu derlemede, CRISPR/Cas9 genom düzenleme sisteminin özellikleri, uygulama alanları ve geleceği hakkında bilgiler özetlenmektedir. Son zamanlarda araştırmacıların amacı, canlı hücrelerin genomunda hedeflenmiş değişiklikler yapmak için uygun maliyetli ve güvenilir yollar geliştirmek olmuştur. Özellikle Çinko Parmak Nükleaz (ZFN) ve Transkripsiyon Aktivatör-Benzeri Efektör Nükleazlar (TALEN) gibi DNA bağlayıcı proteinleri içeren genom düzenleme teknolojileri büyük gelişme sağlamıştır. Biyolojik bilimlerde genomu düzenlemek için hassas ve verimli sistemlere ilave olarak geliştirilen CRISPR/Cas9 sistemi sayesinde, moleküler biyoloji, biyomedikal ve tıp gibi birçok alanda ilerleme kaydedilmiştir. Dahası hemen hemen her organizmanın ve hücre tipinin DNA'sında hedeflenen kırılmalar yoluyla genom mühendisliği alanında önemli gelişmelere katkı sağlamıştır.

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1. Introduction

Along with modifying certain regions of an organism's genetic makeup, treating gene alterations of diseases has always been an interesting area of research. Gene editing methods are used in the therapy of illness caused by many genetic disorders, as well as in the treatment of dissimilar types of cancer (Luthra et al., 2021). Until now, Zinc Finger Nuclease (ZFN), Transcription Activator-Like Effector Nuclease (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) systems have been used as targeted genome editing methods (Gupta et al., 2019). ZFNs and TALENs employ a strategy that binds endonuclease catalytic domains to DNA-binding proteins. It sees DNA double-stranded breaks (DSBs) targeted at specific loci. Cas9, on the other hand, represents a system driven by small RNAs to target DNA (Kaur et al., 2021). Clustered steady spaced short palindromic repeats (CRISPR) were primarily observed in *Escherichia coli* and CRISPR/Cas9 system is a complementary element of prokaryotic adaptable immunity, allowing prokaryotic cells to determine and destroy any stranger DNA (Luthra et al., 2021). They occur naturally in most archaea. They are found in about 40% of bacteria that help degrade DNA or RNA to combat foreign genetic elements such as programmed cell death in eukaryotes (Chandrasekaran et al., 2021).

There are three types of CRISPR/Cas9 systems. So far, most research has been done using the type II system. In this system, the invasive DNA is first divided into small parts and added to the CRISPR locus. The CRISPR space is then transcribed as non-coding pioneer CRISPR RNA (pre-crRNA) and processed as brief strains of mature crRNA. Trans-activating CRISPR mRNA (tracerRNA) jointly with a secondary non-coding RNA forms a ribonucleoprotein complex with the endonuclease Cas9, which sections and recognizes invasive DNA. Cas9 helps with adaptation. Type I and Type II systems are known to objective DNA, while Type III systems target either DNA or RNA. The type II system has been studied more than others due to its capability to induce double-strand breakup in target DNA (Savic and Schwank, 2016; Gupta et al., 2019; Zheng et al., 2020).

The aim of this review is to present the features, applications and future studies of the CRISPR/Cas9 genome editing system.

2. Genome Editing Methods

2.1. Zinc Finger Nuclease (ZFN)

Zinc Finger Nuclease (ZFN) is one of the first genome editing methods to use customizable endonucleases (Martinez-Lage et al., 2017). Zinc finger (ZF) is a protein motif that identifies the DNA sequence and is frequently encountered in transcription factors. It includes two subsites, the DNA binding space and the endonuclease space. Zinc atom is located in the center and is bound to amino acids 19-23 histidine and 3-6 cysteine. Thus, the finger structure is formed (Tufan and Keles, 2019). Amino acids in the zinc finger are able to recognize and bind to a 3-nucleotide sequence by matching with specific DNA sequences (Tufan and Keles, 2019). After the ZF domains bind the DNA target, the

cleaved *Fok*I nuclease domain induces double thread breaks, which are then renovated by non-homologous end-joining (NHEJ) or homology-directed repair (HDR) mechanisms (Figure 1). Although ZFN technology can regulate changes in a particular gene, its use is limited in terms of time consuming and cost because it requires the creation of new ZF proteins, which is technically difficult (Martinez-Lage et al., 2017).

2.2. Transcription Activator-Like Effector Nucleases (TALEN)

The mechanism of action of transcription activator-like effector nucleases (TALEN) is like to that of zinc finger nucleases. However, *Fok*I is used as the cleavage nuclease and TAL proteins are used as the identification strand. TALE proteins were obtained from *Xanthomonas* bacteria that are pathogenic in plants. They consist of DNA contact sites containing a conserved sequence of about 34 amino acids (Figure 1). TALENs are useful because they require less time to form a nuclease than ZFNs and are easy to evolve in terms of specificity and toxicity (Martinez-Lage et al., 2017; Tufan and Keles, 2019).

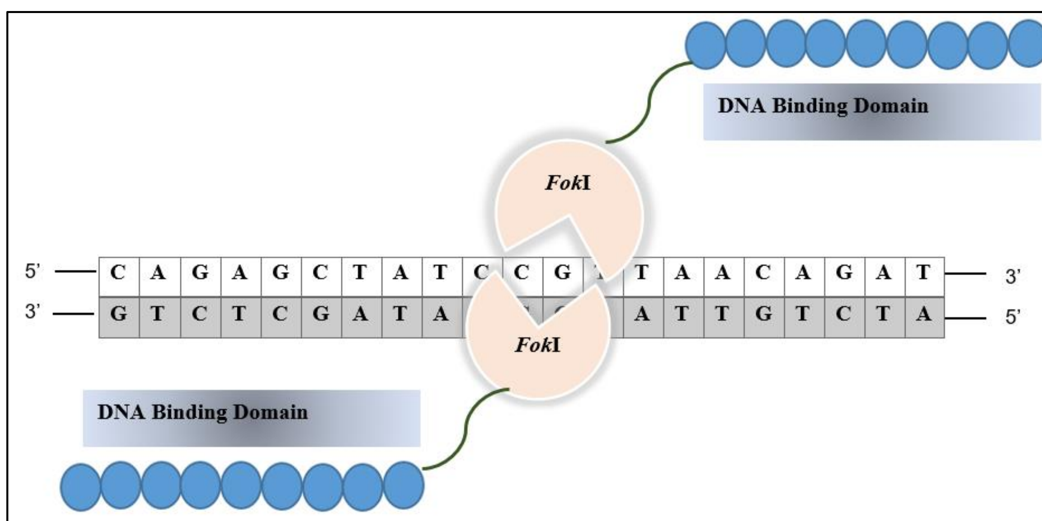

Figure 1. ZFN and TALEN Working Principle (Sakuma and Woltjen, 2014).

Targeted gene editing studies are carried out using ZFN and TALEN systems, known as genome editing technologies. However, their use has been limited for various reasons such as complexity, difficulty and cost. Therefore, researchers have produced a simple, reliable, time-consuming and cost-effective system for genome modification studies. For these studies, and advanced a revolutionary genome regulating technology CRISPR/Cas9 system (Gupta et al., 2019).

2.3. Genome Editing Tool CRISPR/Cas9 System

The CRISPR/Cas9 system is an adaptable immune system that can destroy strange plasmids or bacteriophages found in most bacteria and all archaea. The CRISPR/Cas9 system works with simple RNA-based DNA recognition. It has become an important part of gene editing due to its easy

synthesis and use, low cost and high specificity advantages. It is used as promising application in many fields such as genome engineering, genetic disease diagnosis and high-throughput functionality. The CRISPR system, which first appeared in 1987, consists of the CRISPR sequence with regular repeats and spacing sequences and its associated protein, the *cas* gene (Xie et al., 2021; Wang et al., 2021).

An investigative team led by Jennifer Doudna and Emmanuelle Charpentier was founded in 2012. They adapted the type II CRISPR system from *Streptococcus pyogenes* for genome editing studies. CRISPR/Cas9 is a genome system that purpose to reparation the disease-causing alleles by exchange the existing DNA sequences on the chromosome by becoming a fast and greatly used DNA editing tool in the future. This gene editing system enables genomic editing of species and cell types in many fields of biology, including plants, insects, mice and human cells (Savic and Schwank, 2016; Hryhorowicz et al., 2017).

CRISPR/Cas9 genome system is being studied in 3 stages. Firstly; adaptation or spacer integration, the second is the processing of the principal transcript of the CRISPR steady (pre-crRNA) and maturation of the crRNA inclusive the spacer and the third is the interaction of DNA or RNA and changeable regions corresponding to the 5' and 3' fragments of CRISPR repetitions. The Cas1 and Cas2 proteins establish vast majority of CRISPR/Cas systems are inserted into CRISPR cassettes and form the complex essential for the adaptation process. The endonuclease activity of Cas1 is necessary for conformation integration, while Cas2 is known to perform a non-enzymatic function. The maturation and conversion of pre-crRNA to crRNAs is accomplished by a specific RNA endonuclease complicated or by an alternating mechanism involving bacterial RNase III. The maturing crRNA binds with the Cas protein targeting cognate DNA or RNA (Makarova and Koonin, 2015).

Cas genes encode Cas proteins located close to the CRISPR sequence. The most important feature of these proteins is that they show endonuclease, exonuclease and helicase activities (Ilıkkan, 2020). As a conclusion of the enormous diversity of the CRISPR/Cas system, consistent annotation of Cas proteins has posed great difficulty. Cas proteins are an immensely various group of proteins occurs of almost 45 *Cas* gene families. Cas1 and Cas2 are identical at all CRISPR loci. Cas3, Cas9 and Cas10 are special to type I, type II and type III CRISPR/Cas systems, respectively. All of them contain components necessary for the basic steps of the defensive mechanism (Makarova and Koonin, 2015; Gupta et al., 2019).

CRISPR/Cas9 contains two components for DNA cleavage. Guide RNA (sgRNA), an RNA-guided Cas9 endonuclease and synthetic fusion of crRNA and tracerRNA. DNA separate is inducible by a Cas9-sgRNA ribonucleoprotein complex when the protospacer adjacent motif (PAM) is present and objective DNA sequences match the protospacer area of the sgRNA. The main difference between nucleases of CRISPR/Cas9, such as ZFNs and TALENs, is that CRISPR/Cas9-intermediated DNA split is programmed by a sgRNA. However, ZFNs and TALENs specifically encompass DNA binding sites (Zhang et al., 2021).

3. Components of the CRISPR/Cas9 System

3.1. Endonuclease Cas9

The endonuclease Cas9 is a major multidomain and functional DNA endonuclease that has the ability to shearing the genome at any place (Kozovska et al., 2021). Led by sgRNAs with a 20-nt DNA binding sequence, Cas9 is used as a programmable nuclease tool to modify the DNA sequences in the genomes of many organisms (Xiong et al., 2016).

3.2. Guide RNA (gRNA) or Single Guide RNA (sgRNA)

TracerRNA linking crRNA and Cas9, a sequence-specific targeting tool in most CRISPR-intermediated genome editing systems is combined into a single particle called sgRNA or gRNA. The sgRNA includes a target sequence of 20 nucleotides required to bind Cas9 to a specific genomic site (Kozovska et al., 2021).

3.3. Palindromic Repeat Sequences

The CRISPR locus consists of a CRISPR sequence with specific lengths of DNA repeats, indicated by spacer sequences (Zheng et al., 2020; Panda and Ray, 2022). The length and sequence content of the palindromic repeat sequences at the CRISPR locus are highly conserved, but may still differ between species. Palindromic repeat sequences are sequences that contribute to the creation of the RNA stem-loop secondary structure (hairpin). The repetition zones in these knees can be several or hundreds (Gumus and Kaymaz, 2018). The repeat sequences are very well conserved in the CRISPR region between 21-48 bp (Figure 2) (Gok and Tunalı, 2016).

3.4. Spacer DNA Regions

These sequences, which are highly variable elements of the CRISPR locus, range in length from 26–72 bp. The source of spacer DNAs is the nucleic acids of plasmids or viruses. Spacer DNA sequences can be single or multiple. These spacer DNA sequences constitute the memory of the adaptive immune mechanism in prokaryotes as the essential component of the adaptive immune system (Figure 2) (Gumus and Kaymaz, 2018).

3.5. Leader Sequence

The leader sequence is the starting point of transcription rich in thymine and adenine nucleotides located at the 5' end of the non-coding CRISPR steady. The leader sequence has no open reading frame and is not conserved across species (Figure 2) (Gumus and Kaymaz, 2018).

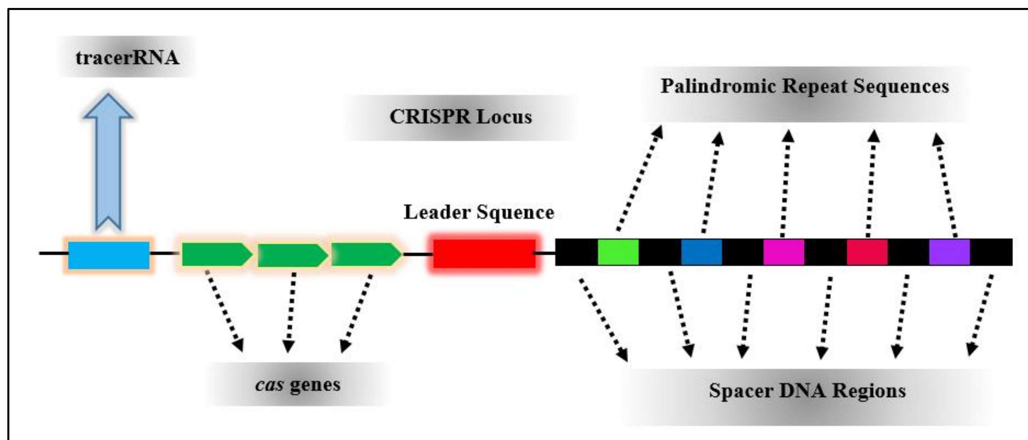

Figure 2. CRISPR Components (Elizondo et al., 2015).

4. CRISPR Applications

4.1. Sickle Cell Anemia

Sickle cell anemia is a hereditary autosomal recessive illness in which valine is located at codon 6 of the β-globin gene instead of glutamic acid. Here, a single amino acid change causes the formation of deoxygenated hemoglobin S (HbS), causing the molecules to appear to stick together. They take on a crescent shape that looks like a sickle when viewed under a microscope. To treat sickle cell anemia, CRISPR-Cas9 uses these two ways: The first is to repair the HbS genes that cause the deterioration of blood cells, and the second is to replace HbS with hemoglobin F (HbF) (Luthra et al., 2021).

4.2. Correction of Genetic Disorders

CRISPR/Cas9 genome system has a significant part in the therapy of genetic irregularity caused by single-gene mutations. Samples of such illnesses contain cystic fibrosis and Duchenne muscular dystrophy (DMD) (Redman et al., 2016). DMD is the most common muscle illness, affecting about 1 in 5000 live male births, reason by mutations of the DMD gene located on the X chromosome.

CRISPR/Cas9 genome editing can improve various DMD mutations and repair functionally disconnected dystrophin evidence in heart and skeletal muscle. Various methods have been utilized for CRISPR/Cas9-mediated DMD disease correction, including exon deletion, skipping and reframing (Zhang et al., 2021). However, adult intestinal stem cells were obtained from patients using CRISPR/Cas9 to investigate the treatment of cystic fibrosis. The mutation reason cystic fibrosis in intestinal organoids has been corrected (Redman et al., 2016).

4.3. Applications in Cell Therapy

CRISPR/Cas9 system has reformed the space of cell therapy. The results of *ex-vivo* gene editing using this therapy on T-cells from patients with cancer or autoimmune disease have been promising. Patient-derived T cells were transferred to patients using the CRISPR/Cas9 system, replacing reapplied stem cells to treat the disease. Also, various activated targets including CXCR4, CCR5, PD-1 and CD7

were delivered to the targeted sequence by electroporation of Cas9 ribonucleoproteins using chimeric antigen receptor (CAR)-T cells. In addition to T cells, pluripotent and hematopoietic stem cells taken from the patient's somatic cells have been applied to regulate the pathophysiological mechanisms of hereditary diseases *ex-vivo* (Redman et al., 2016; Zhang et al., 2018).

4.4. Applications in Pathogenic Diseases

CRISPR/Cas9 is formed from the immune defense system and therefore it has become an advantage to use this system to treat pathogenic diseases. Chronic hepatitis B is known as one of the most common infectious diseases worldwide, which can lead to cirrhosis and cancer. Hepatitis B virus (HBV) is caused by the presence of different closed circular DNAs (cccDNAs) in the liver. In recent studies, CRISPR-Cas9 system targeting cccDNA was used in HBV expressing HepG2 cells. As a result of the study, hepatitis B caused a decrease in core antigen expression and led to the mutation of cccDNAs (Xiao-Jie et al., 2015).

CRISPR/Cas9 system has been applied as an easy and productive technology for genome regulating in establishing animal disease models. For the production of genetically changed mouse models, the CRISPR/Cas9 genome mechanism has been utilized to manipulate genes at the germline or zygote phase. This genome mechanism has been exploited to construct transgenic models of organisms other than mouse. As an example zebrafish codon-optimized Cas9 mRNA and sgRNAs (RNA form) were transferred to single-cell embryos to alter endogenous genes in zebrafish. Also, through the CRISPR/Cas9 genome mechanism, disease-reason mutations in the human hemoglobin beta gene (HBB) from patients with β-thalassemia were eliminated (Xiong et al., 2016).

Back et al. (2019) developed viral vectors and transgenic tools for targeted genome modification in transgenic rat neurons using CRISPR-Cas9 technology. Starting from wild-type rats, they achieved knockdown of tyrosine hydroxylase with adeno-associated viral (AAV) vectors expressing Cas9 or guide DNAs (gRNAs). They created a transgenic rat line to specifically target CRISPR-Cas9 components to dopaminergic neurons, as well as an AAV vector for Cre-dependent gRNA expression. As a result, they developed cell- and sequence-specific genome modifications in the brain tissues of adult rats. Finn et al. (2018) stated that the transthyretin (Ttr) gene was significantly regulated by the application of lipid nanoparticles (LPN) in mouse livers via CRISPR/Cas9-mediated genome editing. According to the results, they obtained a biodegradable and well tolerated LNP delivery system. Thanks to the LNP delivery system, they achieved significant genome editing for high *in vivo* activity levels. Shao et al. (2018) found that hereditary tyrosinemia type I (HTI), a metabolic genetic disorder caused by fumarylacetoacetate hydrolase (FAH) mutation in rats, prevented the progression of liver cirrhosis and liver failure by CRISPR/Cas9-mediated gene therapy.

5. Conclusions and Future Directions

CRISPR/Cas system has been reported to be influential in correction various mutations and restoring protein function at the genomic level. This system provides scientists with the opportunity to fully and quickly gain the desired properties in organisms. ZFNs and TALENs are built on complex, expensive and time-consuming protein engineering.

On the other hand, CRISPR/Cas9 makes it possible to correct errors in the genome simultaneously in multiple independent regions that are shortly programmable, inexpensive for DNA targeting, easy to design and manufacture. It also provides an alternative to ZFN and TALEN in genome editing.

Recently, CRISPR/Cas9 system has made important progress in genome regulating. However, despite the challenges, the CRISPR/Cas9 system remains an effective, precision and accurate guide for genomic manipulation. It is assumed to continue to be used as a tool with the effective use of functional genomic studies by becoming more sensitive and efficient day by day.

CRISPR/Cas9 will be a glimmer of hope in the treatment of many deadly diseases such as cancer with therapeutic adjustments in the future. CRISPR/Cas9 will also provide significant advances in the pharmaceutical industry and will enable the development of new drugs using genetic methods in the treatment of diseases known to be deadly. Thanks to gene editing systems, serious measures can be taken against the release of organisms that will cause ecological damage and affect human health. Moreover, by using CRISPR/Cas9 gene editing technology, environmental stress tolerance and disease resistance of plants could be improved.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The contribution of the authors is equal.

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