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The CRISPR/Cas system: Gene Editing by bacterial defense

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Abstract

The most precise, effective, and widely used tool for editing the genome is currently the clustered regularly interspaced short palindromic repeat (CRISPR), which represents the prokaryotes adaptive immune defense. The CRISPR/Cas-9 genome editing system relies on two key elements; mainly the guide RNA (gRNA) and CRISPR-associated (*Cas-9*) proteins. A complementary base pair in the designed sgRNA allows it to recognize the target sequence in the gene of interest. Either the non-homologous end joining or the homology-directed repair can be used to repair the double-stranded breaks, which are created by *Cas-9* nuclease at a position upstream from a protospacer adjacent motif. The modified genome-editing tool CRISPR/Cas-9 has numerous applications in the various fields, such as biotechnology and medicine. Moreover, it is being studied for cancer management; Human Immunodeficiency virus (HIV), and as a gene therapy for several genetic diseases, including cystic fibrosis; sickle cell disease, and Duchenne muscular dystrophy. However, immunogenicity; off-target effect, and efficient delivery systems withstand against its spread in the clinical applications until introducing an improvement. The aim of this review was to summarize how the various CRISPR systems work; their important medical applications, and their limitations.

Keywords: CRISPR, *Cas-9*, gRNA, Gene editing

1. Introduction

In 1987, a Japanese research team identified five homologous 24 nucleotide sequences that were separated by 32 nucleotide spacers, and this was the beginning of the fascinating story of the Clustered Regularly Interspaced Palindromic Repeats (CRISPR). However, this team was unable to fully explain the biological implications of their discovered sequences (Ishino *et al*., 1987). Later, other research teams gradually identified similar arrays of repetitive sequences with regular spacing, thus revealing their biological relevance not only as a prokaryotic adaptive defense, but also as a genetic therapeutic tool (Jore *et al*., 2012).

The CRISPR/Cas system stores a memory of phage DNA in the chromosomes of the bacterial hosts to guard against their similar future viral infections; hence expressing a form of the prokaryotes adaptive immunity. This system contains CRISPR genomic locus that harbors direct repeats, which are short repetitive nucleotide sequences surrounding the phage genetic element. Near their end; these repetitions are flanked by CRISPR associated (*Cas*) genes sequences that code for the *Cas* protein family (Barrangou *et al*., 2007; Ahumada-Ayala *et al*., 2023). The CRISPR system; known as a molecular scissor or a gene scissor, is currently one method of DNA and RNA editing technique, which alters the genome in a sequence-dependent way using artificially guided reprogrammed endonucleases that are directed toward the target gene (Li *et al*., 2022).

The objective of this review was to summarize the key findings in the era of CRISPR-Cas; explaining how various CRISPR systems work, their important medical applications, and their limitations.

2. Timeline of the CRISPR/Cas system

2.1. Identification scene (1987-1993)

The genome of *Escherichia coli* represents the first spot for the discovery of five directly repeated 24 nucleotide sequences of the CRISPR/Cas system (Fig. 1) (Ishino *et al*., 1987). *Haloferax archaea* showed the second scene with 30-34 nucleotide sequences direct repeats separated by spacers (Mojica *et al*., 1993). Between 1987 and 1993, the scientists were fascinated by the sequences that the CRISPR/Cas system used as spacers or direct repetitions.

2.2. Structural and functional description scene (1993-2011)

By the year 2000 and among twenty different microbial species; Mojica's team had discovered the CRISPR-DNA fragments (Fig. 1) (Mojica *et al*., 2000). In 2002, the term CRISPR was coined to describe the interspaced repeat arrays that were detected in the microbial genomic loci with the identification of their nearby genes; mainly *Cas* genes (Jansen *et al*., 2002). Meanwhile, in 2005, the foreign extra-chromosomal origin of the spacers was linked to the bacteriophages (Mojica *et al*., 2005). It was established in 2007 that the CRISPR/Cas system enabled the prokaryotes adaptive immunity against the bacteriophages; through matching the genomic sequence in the spacer that was stored in the host genome and the phages (Barrangou *et al*., 2007). In 2011; an expanded investigation of the phylogenetic linkages between CRISPR and *Cas* proteins was presented by Makarova *et al*., (2011).

2.3. Implementation scene (2012-present)

In 2012, CRISPR was established as a gene-editing technology because of its superior potential to cleave the targeted DNA segments beside its easy design for the researchers, which speeded up its widespread adoption (Fig. 1) (Jinek *et al*., 2012; Cho *et al*., 2013; Guo *et al*., 2022). CRISPR/Cas-9 gene editing technology is currently a common and flexible tool used to alter the genomes of a variety of cells in the fields of human gene therapy and synthetic biology. In China, the CRISPR/Cas-9 was first implicated clinically for the treatment of lung cancer in 2016. Many CRISPR/Cas9-based clinical treatments have been reported in the last years (Nidhi *et al*., 2021).

2.4. Nobel Prize

CRISPR is currently regarded as the most significant molecular biology discovery since polymerase chain reaction (PCR). In 2020, the development of CRISPR/Cas genome editing techniques earned Emmanuelle Charpentier and Jennifer Doudna the Nobel Prize in Chemistry in appreciation of their work (Mosterd *et al*., 2021).

3. Functional mechanisms of the CRISPR/Cas system

a. Adaptation

Adaptation is the process of incorporating a unique foreign DNA sequence (protospacer) into CRISPR

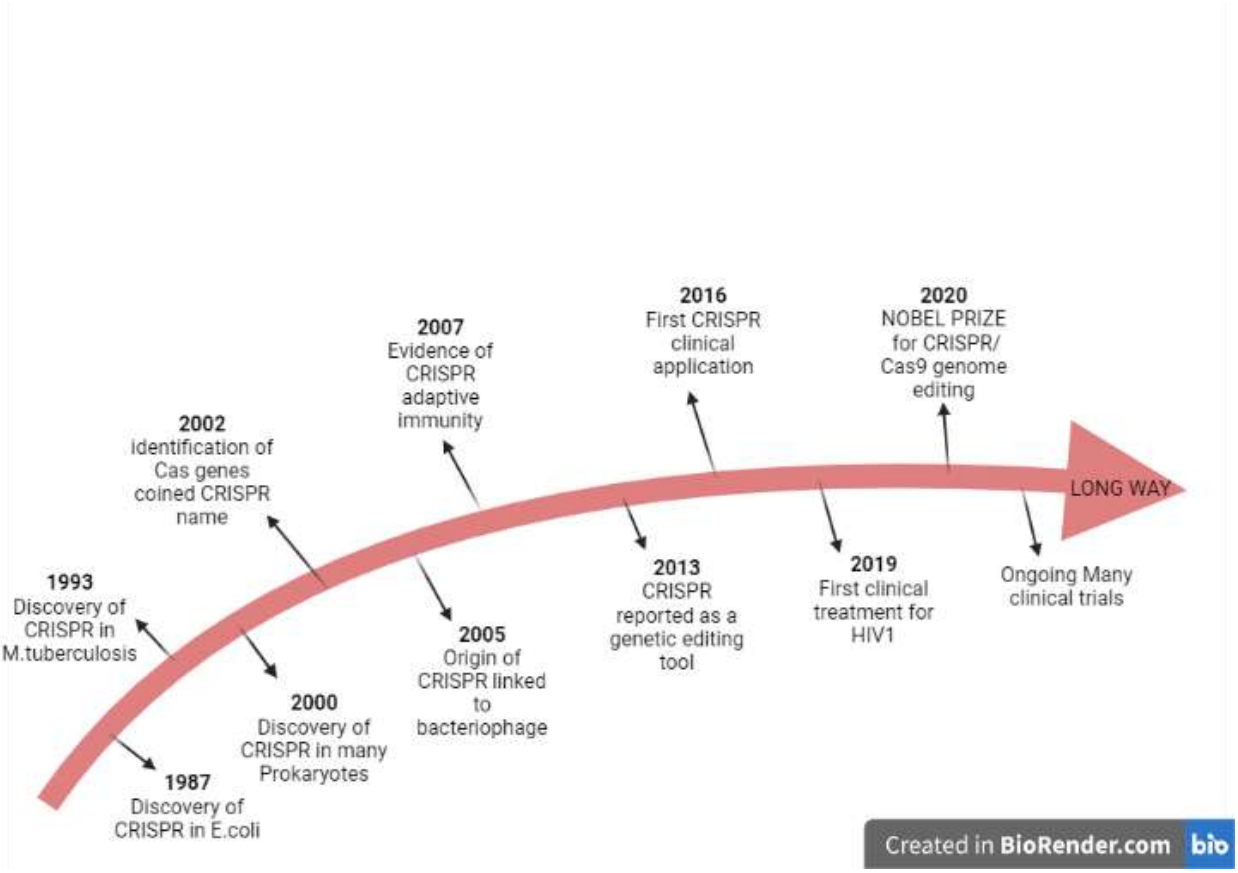

Fig. 1. Timeline of the (CRISPR)/Cas system (Created with BioRender.com, accessed on 2 March, 2023)

arrays guided by their leader sequences (A-T rich region) after a repeat sequence is duplicated; thus generating a novel spacer between the two repeats. It is also referred to as insertion or acquisition that takes place only when the protospacer adjacent motif (PAM); a sequence surrounding the protospacer, is recognized (Hille and Charpentier, 2016). This demonstrates the adaptive function of the immune system, which allows the genetic information of the attacker to be memorized by the host (McGinn and Marraffini, 2019).

b. Expression of CRISPR RNA (crRNA) biogenesis

Expression refers to the transcription of CRISPR arrays into CRISPR RNA precursors (pre-crRNA) together with the expression of additional *Cas* genes. The pre-crRNA is further processed by being cut off to produce mature and shorter crRNAs with the aid of some expressed *Cas* proteins (Makarova *et al*., 2020). For the pre-crRNA to be processed in type II systems; trans-activating CRISPR RNA (tracrRNA) in duplex with the crRNA is necessary, to direct *Cas9* to its target DNA (McGinn and Marraffini, 2019; Li *et al*., 2023).

c. Interference

This process involves the specific interference with the invading virus or the plasmid nucleic acids using mature crRNAs as a guide. The target degradation is achieved in class 1 systems via a multi-protein effector

complex consisting of multiple *Cas* proteins and crRNA. However, the target interference in class 2 systems just requires a single effector *Cas* protein plus crRNA. Cleavage and inactivation of the invading genome occurs after recognition and binding of *Cas* proteins to the PAM (Karginov and Hannon, 2010).

4. CRISPR locus structure

The three main components of the CRISPR locus are demonstrated in Fig. (2) as follows:

-The CRISPR locus is defined as a collection of short direct repeats of identical lengths and sequences that are interrupted by spacers.

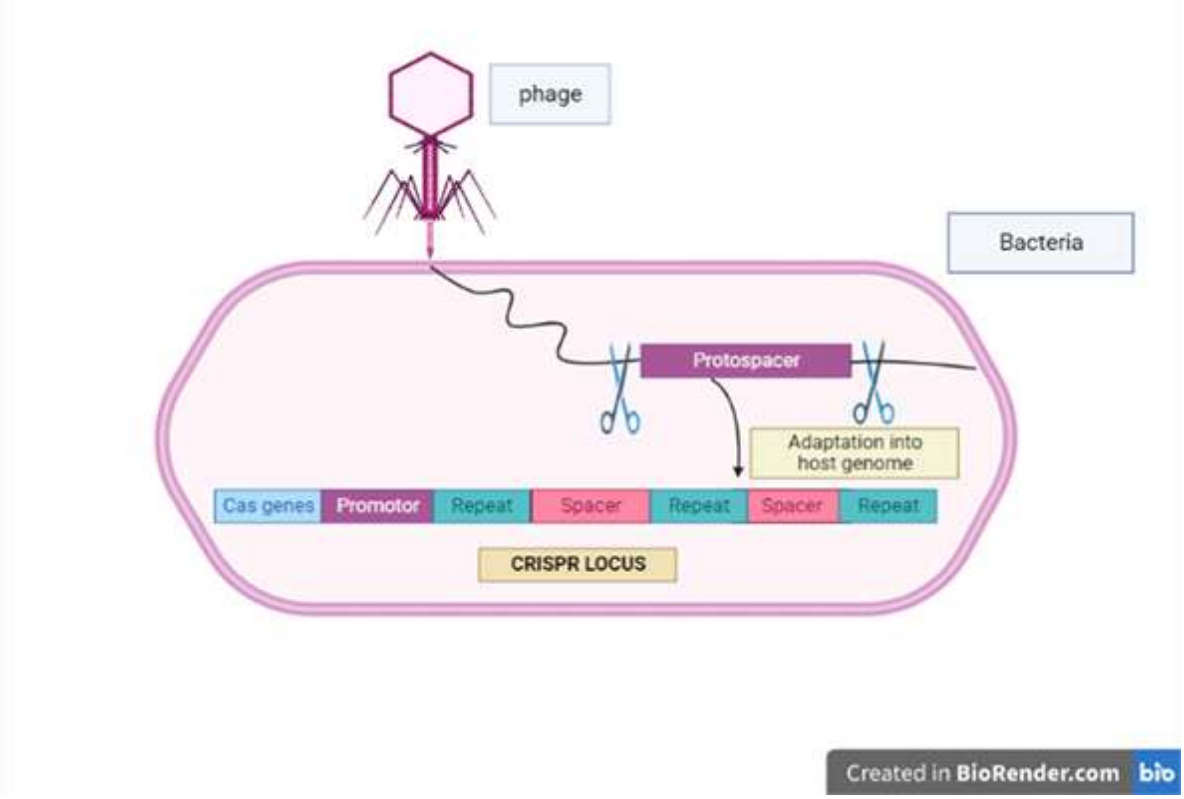

Fig. 2. Structure of CRISPR locus (Created with BioRender.com, accessed on 28 February, 2023)

The repeats are typically 32 bp in size, while the spacers have fixed nucleotide sequence lengths that range from 20 to 72 bp within the various species. The related species may share similar repeat sequences; although there is a great diversity of spacers and repeats among the bacteria and archaea (Mosterd *et al*., 2021).

-The leader sequence, which plays a role in the transcription and adaptation; is frequently observed in a specific orientation on one side of the CRISPR array.

Little sequence conservation is detected in these regions (Kurihara *et al*., 2022).

-The *Cas* genes; those genes that code for the related *Cas* proteins are arranged differently. The *Cas* proteins are crucial for both getting and removing the foreign sequences (Mosterd *et al*., 2021).

5. Classification of the CRISPR/ Cas system

There are 2 main classes of the CRISPR/Cas system, Class 1 and Class 2; where both are divided into several subtypes (Table 1). Type I, III, and Type IV systems comprise the Class 1 system; where the interference step is carried out *via* an effector complex made up of several *Cas* proteins and crRNA (Makarova *et al*., 2020; Bhatia *et al*., 2023).

The type I system has seven subtypes (A-G), including various variants. The pre-crRNA is typically cleaved in type I system by the *Cas6* protein. The CRISPR-associated complex (Cascade complex) is the effector complex for the antiviral interference phase in type I systems. Diverse subtype's assembly of *Cas3*; *Cas5*, *Cas7*, *Cas8*, and other *Cas* proteins make up the Cascade complex; with *Cas3* protein representing the key element for cleavage (Karginov and Hannon, 2010).

The type III system involves 6 subtypes (A-F); where *Cas6* protein also mediates the pre-crRNA cleavage. *Csm* and *Cmr* complexes; consisting of corresponding *Csm* or *Cmr*/ Cas proteins and crRNA, characterize the effector complex for this type. Type IV system that lacks adaptation has three subtypes (A-C) with *Cas6* protein acting as a crRNA processor; where large subunits *Csf1*; *Cas5*, and *Cas7* proteins form the effector complex (Makarova *et al*., 2020; Mosterd *et al*., 2021).

The effector complex in Class 2 system types (II, V, and VI) is a single *Cas* protein that is bound to crRNA. To process the pre-crRNA in type II systems: tracrRNA; *Cas9* protein, and RNase III collaborate with the *Cas9* as the only effector protein for cleavage of the target sequence, thus producing blunt ends. The *Cas12* and *Cas13* proteins function as the effector complexes and pre-crRNA processors in Type V and Type VI systems, respectively (Hille *et al*., 2018; Zhou *et al*., 2021).

6. Mechanism of CRISPR/Cas9-mediated genome editing

Type II CRISPR/Cas-9 has been extensively employed in genetic engineering since its structure is reasonably simple. It has two important elements; mainly guide RNA (gRNA) and *Cas-9* proteins. *Streptococcus pyogenes* provides the first *Cas-9* protein used in genome editing (SpCas-9), which is classified as a Class 2, Type II, subtype IIA. Type II CRISPR/Cas-9 is termed as the genetic scissor that creates target DNA double-stranded break (DSB), which is repaired by the host cellular machine through its endonuclease activity (Asmamaw and Zawdie, 2021).

Cas-9 is formed of 2 lobes, including the recognition (REC) and the nuclease (NUC) lobes. The REC lobe binds the guide RNA. The NUC lobe that is composed of RuvC; HNH, and PI interaction domains is responsible for DNA cleavage and PAM specificity. As demonstrated in Fig. (3), both crRNA that pair with the target sequence and tracrRNA, which provide a scaffold for *Cas-9* nuclease, form guide RNA (Uddin *et al*., 2020).

As a gene-editing tool, a single guide RNA (sgRNA) can be synthesized by combining the crRNA; tracrRNA and artificial RNA linker to a target; nearly any gene sequence that is intended to be edited (Mosterd *et al*., 2021).

The editing system uses the homology-directed repair (HDR) and the non-homologous end joining (NHEJ) pathways as its two primary mechanisms for repairing the DSBs created by *Cas-9*. NHEJ helps the DSB repair by joining the DNA fragments in the absence of an exogenous homologous DNA. Although NHEJ is the most prevalent and effective repairing process, it can produce frame shift mutations resulting from random insertions or deletions at the break site;

Table 1: Characteristics of the various CRISPR systems (Xu and Li, 2020)

		Target	Effector complex	Cleavage protein	Characters
Class 1	Type I	DNA	*Cas7*, *Cas5*, *Cas8*	*Cas3*	Cleaves ssDNA strands
	Type III	DNA/ RNA	*Cas7*, *Cas5*, *Cas10*	*Cas10*	Binds to nascent RNA molecules
	Type IV	DNA	*Cas7*, *Cas5*, *Csf1*	---	Most unknown
Class 2	Type II	DNA	*Cas9*	*Cas9*	Originates blunt DSB
	Type V	DNA	*Cas12*	*Cas12*	Originates staggered DSB
	Type VI	RNA	*Cas13*	*Cas13*	RNA-guided RNase

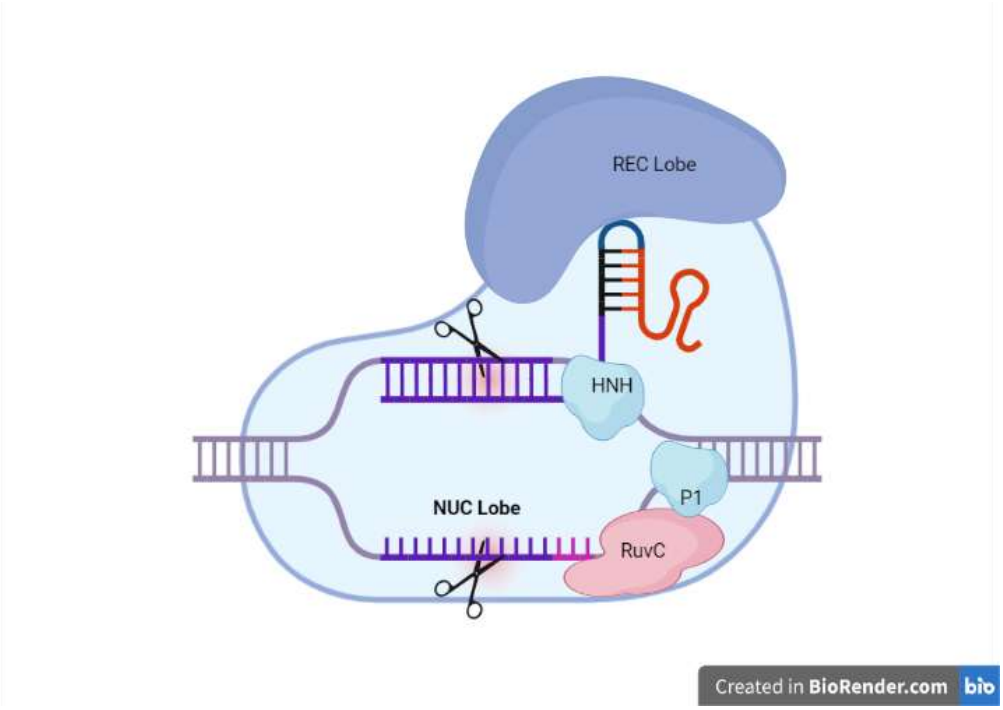

Fig. 3. Mechanism of CRISPR/Cas9-mediated genome editing (Created with BioRender.com, accessed on 27 February, 2023)

yielding premature stop codons or non-functioning protein. Hence, a gene therapy can knockout any unwanted gene using the CRISPR/Cas9 system (Zhang, 2021). The error free HDR uses a homologous unedited transferred DNA strand as a template to insert the desired DNA sequence into the genome after repairing the DSB, so it is preferred for the therapeutic applications. To do this, a repair template with ends that match the upstream and downstream region of the DSB is created to anneal to each side of the break. So the HDR can perform the knock-in (insertion) or knock-out (replacement) techniques (Fig. 4) (Uddin *et al*., 2020; Zhang, 2021).

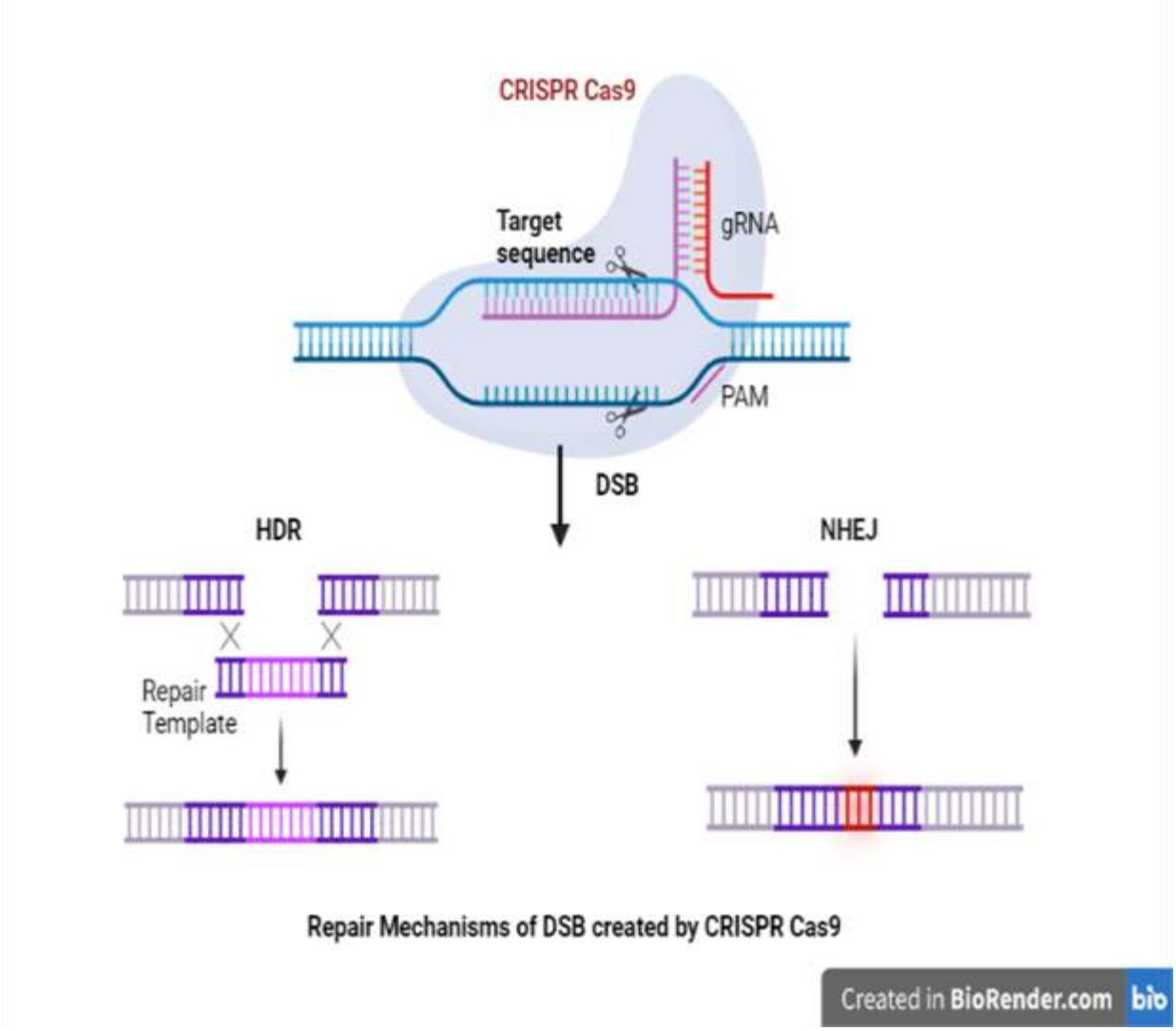

Fig. 4. Repair mechanisms for DSBs created by *Cas-9* (Created with BioRender.com, accessed on 27 February, 2023)

Base editing is also a type of genome editing technique introduced by CRISPR/Cas9. It produces DNA or RNA point mutations without causing DSBs; through the modification of *Cas* endonuclease to deactivate the HNH and RuvC. The two categories of DNA base editors fused with *d/nCas9* are the cytosine base editors (CBEs) and adenine base editors (ABEs). The ABEs change the A-T/G-C base pair, whereas the CBEs change the C-G/T-A base pair (Sen *et al*., 2022).

A recent use of the CRISPR/Cas genome editing technology is the prime editing; where two significant alterations are made to the conventional CRISPR/Cas9 system. Initially, a reverse transcriptase is coupled to *Cas9*. In addition, RNA sequence with a primer binding site (PBS) complementary to the edited DNA sequence becomes connected to the gRNA; thus producing a primary editing guide RNA (pegRNA) (Asmamaw and Zawdie, 2021). The prime editing uses the broken DNA as a primer to produce a DNA fragment with the new edited sequence without the need for a donor DNA template (Sen *et al*., 2022).

7. Applications of CRISPR/CAS-9

7.1. Role in gene therapy

The CRISPR/Cas9 RNA-guided genome editing tool, which is recently added to the list of the programmable nucleases; has a number of benefits over the conventional protein-guided ones, including the zinc finger nucleases (ZFNs) and the transcription activator-like effector nucleases (TALENs). First, CRISPR/Cas9 requires simply the design of a complementary sgRNA with the same nuclease *Cas9* in all circumstances compared to ZFN or TALEN, which require *de novo* production of a complex guiding protein. Second, CRISPR/Cas9 has a multiplexing advantage as it can target different genomic loci with multiple sgRNAs that edit them in parallel (Xiao-Jie *et al*., 2015). Third, it has multifunctional programmability, as it can insert, delete or repair genes. All of these advantages are accompanied with its high potency and specificity; broad applicability *in vivo* and *ex vivo*, and low cost (Zhang, 2021).

The promise of CRISPR/Cas-9 gene editing is to treat the majority of the known hereditary illnesses, including sickle cell disease (SCD); β-thalassemia, cystic fibrosis, and muscular dystrophy (Khosravi *et al*., 2019).

7.2. Management of cancer

Cancer is characterized by many oncogene and epigenetic mutations that open up the possibility of using gene therapy to treat this disease. The rapid advancement of CRISPR/Cas9 genome editing technology is shifting it to be a powerful tool for efficiently modifying a specific gene sequence, and thus making it a promising therapy for cancer treatment. Several clinical trials of using CRISPR/Cas9 in treatment of cancer and other genetic diseases are listed in Table (2) (Sen *et al*., 2022).

In 2019, the University of Pennsylvania began the first research to look into a CRISPR-created cancer treatment in the United States. The therapy entails creating four genetic changes to the T cells to kill the cancer cells named NYCET. The CRISPR/Cas9 has been first tested in treatment of the lung cancer, followed by advanced multiple myeloma and metastatic sarcoma (Baylis and McLeod, 2017).

7.3. Control of several infectious diseases

7.3.1. Control of human immunodeficiency virus (HIV)

CRISPR/Cas technology has also demonstrated promising potential in the treatment of Human Immunodeficiency virus (HIV). CRISPR/Cas9 has been used to reduce the HIV-1 infection through targeting the long terminal repeat (LTR) regions of the viral genes into HIV-1 inducible T cells; with subsequent decrease in expression (Hu et al., 2014). Moreover, genome editing of the cellular cofactors; mainly chemokine receptor type 4 (CXCR4) and

Table 2: List of ongoing CRISPR/Cas clinical studies for treating various diseases (Sen *et al*., 2022)

Target disease	Editing procedure	References
HIV	LTR U3 region (Gene knockout)-Loss of LTR expression CCR5 on T cells (Gene knockout)-Reduced virus entry	(Hu *et al*., 2014) (Hou *et al*., 2015)
HBV	Repeat regions (Gene inactivation)	(Luthra *et al*., 2021)
Sickle cell disease	BCL11A (Gene interference) HBB (Indels)	(Khosravi *et al*., 2019)
β -thalassemia	HBB (Mutation deletion)	(Akinsheye *et al*., 2011)
Non-small cell lung cancer	PD-1 (Gene knockout)	(Baylis and McLeod, 2017)
Cystic fibrosis	CFTR (Base editing)	(Maule *et al*., 2020)
Duchenne muscular dystrophy	Dmd (Gene deletion)	(Fortunato *et al*., 2021)
Huntington disease	HTT (Small targeted Deletion)	(Luthra *et al*., 2021)
Alpha 1-antitrypsin	SERPINA1 Gene disruption	(Wu *et al*., 2020)
B-Cell Leukemia and Lymphoma	CRISPR/Cas9-engineered CAR-T-cells to target CD19 and CD20/CD22	(Manguso *et al*., 2017)
B-Cell Non-Hodgkin's Lymphoma	CRISPR-engineered allogeneic Anti-CD19 CAR-T-cell therapy	(Manguso *et al*., 2017)
Advanced Hepatocellular Carcinoma	PD-1 knocked-out CRISPR-engineered T-cells	(Luthra *et al*., 2021)
Multiple myeloma, Sarcoma, Melanoma	Engineered T-cells using CRISPR for expressing NY-ESO-1 TCR	(Baylis and McLeod, 2017)
Cancer (Gastrointestinal, colorectal, pancreatic, gallbladder, esophageal, stomach)	engineered T-cells using CRISPR/Cas9 to inhibit genes which encode for CISH (an intra-cellular checkpoint target)	(Luthra *et al*., 2021)
Epstein-Barr virus related malignancies	PD-1 knocked-out CRISPR-engineered T-cells	(Nidhi *et al*., 2021)

Where; LTR: Long terminal repeat, CCR5: Chemokine receptor type 5, BCL11A: B-cell lymphoma 11A, PD-1: Programmed death-1, CFTR: Cystic fibrosis trans-membrane conductance regulator, Dmd: Duchenne muscular dystrophy, HTT: Huntingtin protein-coding, SERPINA: Serine protease inhibitor, CAR: Chimeric antigen receptor

chemokine receptor type 5 (CCR5) by CRISPR/Cas9 showed more resistance to HIV-1; through blocking the viral entry into the edited cells (Hou *et al*., 2015).

7.3.2. Control of other viral diseases

Because of their intracellular position and their need for protein substrates to replicate, viruses are

obvious candidates for genetic alterations using CRISPR. This technology can be used to target the viral regions; rendering the viruses incapable of transcription or replication. As a result, CRISPR gene therapy appears to be a promising treatment for such complex viral infections characterized by high mutations and latencies (Mosterd *et al*., 2021).

A designed base editing *Cas9* protein is used to target the Hepatitis B (HBV) virus by changing the nucleotides; resulting in a nonsense codon that prevents the viral proteins from being translated. This technology is also reported to be efficiently attacking the other human DNA viruses. Apart from DNA targeting *Cas9*; the RNA targeting CRISPR-Cas13 is an antiviral strategy used in the human cells against the ssRNA viruses (Luthra *et al*., 2021).

7.3.3. CRISPR in COVID-19

A CRISPR-based technique is being searched as an alternative antiviral tool for recognizing and eliminating the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within the cells. CRISPR-Cas13 is used in employing the sequence-specific crRNAs targeting the SARS-CoV-2 ssRNA genome; *ORF1ab*, and *S* genes (Nguyen *et al*., 2020).

7.3.4. Control of bacterial infections

The public health experts are currently most concerned about the increasing number of antibiotic resistant bacteria caused by the misuse or overuse of antibiotics; calling for the development of new strategies to combat these bacterial infections. By blocking the genes that cause antibiotic resistance or by cleaving the key regions of a bacterial genome; CRISPR systems can be utilized in conjunction with the traditional antibiotics. *Cas9* has been used to target the β-lactamase and kanamycin resistance genes in *E. coli* and *Staphylococcus aureus*; respectively, with high efficiency (Loureiro and da Silva, 2019).

8. Difficulties for application of CRISPR/Cas-9 and the potential solutions

Despite its immense promise as a genome-editing system, CRISPR/Cas-9 technology has been impeded by various obstacles, including immunogenicity, lack of a safe and effective delivery system to the target, and off-target effect, which should be considered during the application process (Xiao-Jie et al., 2015).

8.1. Immunogenic response

The host immunity may cause an immunological reaction to the CRISPR/Cas-9 system components because they are of bacterial origin. The *Cas-9* protein has already elicited humoral and cellular (*i.e*., anti-*Cas-9* Ab and T cells) immune responses. Currently, finding a way to identify and reduce the immunogenicity of *Cas-9* protein is one of the main challenges in the clinical study of this system (Asmamaw and Zawdie, 2021).

8.2. Delivery of CRISPR gene therapy

The delivery of the CRISPR/Cas9 system into the cells has so far shown to be a major challenge to overcome before significant *in vivo* utilization of this system can become a reality. For CRISPR gene editing to function; effective and safe delivering of its components into the cell is essential (Loureiro and da Silva, 2019).

8.3. Off-target effects

The primary cause for confirmation of the off-target effects is the failure of sgRNA protospacer sequence to recognize the target sequences; leading to unexpected genetic alterations such as mutation; deletion and oncogene activation, which limit the therapeutic uses of the CRISPR/Cas-9 editing system (Cho *et al*., 2014).

Many approaches have been created to reduce the probability of the CRISPR/Cas-9 off-target effects. First, proper design of the sgRNA for the targeted DNA with a GC content between 40 % and 60 %; applying a truncated sgRNA to decrease the mismatch, and selecting a target region that has no homologous sequence in the genome. Second, *Cas-9* nuclease can

be mutated into a nickase by changing either of its catalytic residues (*i.e*., HNH or RuvC), which can only produce a single-stranded break rather than a DSB. This double-nicks approach is reported to decrease the off-target effects by 1500 times. Third, peptide delivery of *Cas9* has been shown to cause a lower off-target activity. Finally, the CRISPR-Cas9 in combination with other nucleases such as CRISPR/Cas-12a may be helpful; as the *Cas-12a* can produce crRNA from the pre-crRNA without a need for tracrRNA, thus decreasing the plasmid vector size (Zhang, 2021).

9. Modifications of the CRISPR-Cas system

Researchers are working to enhance the CRISPR systems efficiency and consistency despite the pre-mentioned drawbacks; through the introduction of novel *Cas* elements, including:

Cas12a

As opposed to *Cas9*, *Cas12a* requires a crRNA molecule as a guide towards its target; without the tracrRNA. It allows multiplex gene editing through introducing a single pre-crRNA template into the cell, which is then cleaved by the *Cas12a* into several crRNA molecules. Furthermore, the DNA-DSBs produced by *Cas12a* become staggered, which are preferably repaired through HDR rather than NHEJ; leading to a lower mismatch and off-target effects (Zetsche *et al*., 2017).

Cas13a

Similarly, as *Cas12a*, the *Cas13a* can perform its own crRNA processing; so that it becomes able to target numerous loci using a single pre-crRNA template. In addition, the mRNA can be a target for *Cas13a*, which is helpful for treating many diseases, through the action of its two HEPN domains on the RNA leading thus to its cleavage (Cho *et al*., 2014).

Cas9n

Nickase Cas9 (*nCas9*); a frequently used modified variation of the CRISPR systems, has been developed by causing a point mutation in one of the *Cas9* cleavage domains. This mutation interferes with RuvC or HNH cleavage ability; causing a single genomic nick on the desired DNA strand. The base excision repair is the preferred method for repairing the single nicked DNA. Therefore, *Cas9n* can be utilized to increase the effectiveness of the procedure by lowering the amount of mutations, which are brought on by the unintended NHEJ repairs (Zhang, 2021; Khoshandam *et al*., 2023).

dCas9

When *Cas9* RuvC and HNH catalytic domains undergo two silencing mutations that render them inactive, the CRISPR system loses its ability to cleave the DNA; however, it keeps its capacity to attach to the specific sequences forming a dead *Cas9* protein (*dCas9*). This catalytically inactive *dCas9* can inhibit the transcription by preventing pairing of the RNA-polymerase with the promoter sequences that the *dCas9* targets (Cho *et al*., 2014).

Conclusion

In the present era, the most advanced biotechnological tool is the CRISPR-Cas, which represents the prokaryotes natural and adaptive defense against the viruses by storing bits of the viral DNA in a way that catalogue the different viral infections to which the bacteria are exposed to throughout their life. The CRISPR/Cas-9 system has two elements; mainly the guide RNA for editing the target gene and *Cas-9*, which efficiently cuts the DNA at the spot specified by the guide RNA. For the ambitious uses, the CRISPR-Cas system still needs to be safe by minimizing the off-target effects and overcoming the delivery issues. However, it is considered as a very promising era in the molecular biology, which is effectively used in gene therapy with the ease of programing.

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Conflicts of interest

The author declares non-existence of any conflict of interests.

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Author Contribution

The author fully contributed in this study.

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