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Review Article

CRISPR/Cas9-Gene Editing Technology

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Abstract

The development of the *CRISPR*/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/*CRISPR*-associated protein 9) eukaryotic gene editing technique opened up new possibilities for therapeutic interventions as well as for the study of gene function. Although targeted gene disruption was possible using the original methods, current technical developments have produced a wide range of instruments that can change genes and gene expression in many different ways. In this manner, the researchers modified the bacteria's resistance to a particular virus, proving the significance of *CRISPR*s in regulating bacterial immunity. The components of *CRISPR* as a molecular tool can be delivered through various techniques, despite the fact that the functional *CRISPR* in the nucleus is in RNP form. The *CRISPR*/Cas methods have further enhanced functional capabilities with reduced off-target effects.

Keywords: *CRISPR*/Cas9; Gene editing; Non-homologous end joining; Homology-dependent repair; Gene knockout; Gene knockdown

Introduction

Clustered, regularly interspaced palindromic repeats (*CRISPR*)/Cas9 are a gene-editing technology used in various research fields, including the health and veterinary sectors. It makes it feasible to swiftly, affordably, and relatively simply change the expression of genes in cells and organisms as well as rectify faults in the genome. It has several laboratory uses, such as the quick creation of cellular and animal models, functional genomics assays, and real-time cellular genome imaging [1].

It has been demonstrated that it is possible to utilise it to repair damaged DNA in mice, curing them of genetic defects, and it has been hypothesised that human embryos can be similarly transformed. Additional possible clinical applications include gene therapy, the treatment of infectious diseases like HIV, and engineering autologous patient material to treat cancer and other diseases [2]. This article will also describe some of the difficulties and ethical controversies associated with this novel technology (Figure 1) [3].

Overview of *CRISPR*/Cas9

CRISPR/Cas9 is a gene-editing technology which involves two essential components: a guide RNA to match a desired target gene and Cas9 (*CRISPR*-associated protein 9) an endonuclease which causes a double-stranded DNA breaks, allowing modifications to the genome.

***CRISPR*s:** The term "Clusters of Regularly Interspaced Short Palindromic Repeats" (*CRISPR*) refers to a region of DNA made up of brief, repetitive sequences with so-called "spacers" inserted in between each repeat. The rungs that make up a DNA molecule's spiral staircase are referred to as repeats in the genetic code. Each rung is made up of two rungs: Adenine and Thymine (A and T), and Guanine and Cytosine (G and C). Bases in the *CRISPR* region can be found in "palindromic" sequences, which are repeated several times in the same order. The nucleotides on one side of the DNA ladder that are read in the opposite direction match those on the opposite side in a palindromic pattern. The DNA of the *CRISPR* system is composed of short palindromic repeats, each of which is surrounded by "spacers." In order to incorporate some viral DNA into their own genome, bacteria take these spacers from viruses that have infected them [4].

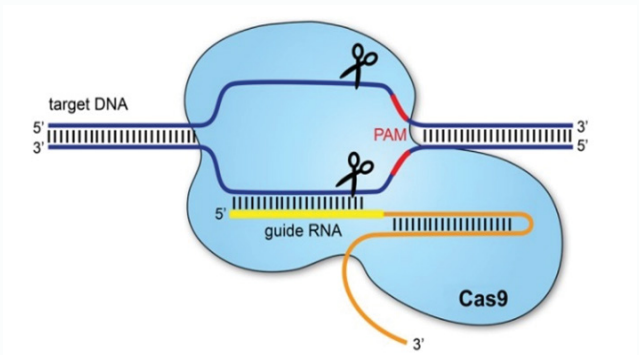

Figure 1: How CRISPR/Cas9 works [3].

In the event that the viruses recur, the bacteria will be able to identify them thanks to the memory bank that these spacers serve as. Spacers can also be compared to "Wanted" signs because they show a clear picture of the culprits, making it easier to catch them and punish them. Rodolphe Barrangou and a group of researchers at the food additives producer Danisco first experimentally validated this process [5]. The bacteria were seen to add more spacers to their *CRISPR* regions following a viral infection. Furthermore, these spacers' DNA sequences matched that of some virus genome segments. The team

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also deleted the spacers and replaced them with new viral DNA sequences. Scientists are able to manipulate the bacteria's resistance to a particular virus, which is proving to be a sign of *CRISPR*s in regulating the immunity of bacteria [6].

***CRISPR* RNA (crRNA):** Viral information is stored in *CRISPR* regions of DNA, but before this data can be used elsewhere in the cell, it must be duplicated, or "transcribed," into RNA. Unlike DNA sequences, which remain embedded within the DNA molecule, this *CRISPR* RNA (crRNA) may move around the cell and work with proteins, namely the molecular scissors that cut viruses into pieces. In contrast to DNA, which has two strands, RNA only has one, giving it the appearance of a partial ladder. In order to create an RNA molecule, one component of *CRISPR* acts as a template. Proteins called polymerases then build an RNA molecule that is "complementary" to the template, which means that the bases of the two strands fit together like jigsaw puzzle pieces. An example would be the translation of a G in the DNA molecule into a C in the RNA [7].

Cas9: Cas9 is an enzyme that is capable of severing foreign DNA. The protein binds to the RNAs cr and trac, which together help Cas9 to cut the region of the viral DNA strand. A "nucleotide" is a single-base DNA-building component, and Cas9 will sever target DNA that is complementary to a crRNA that is 20 nucleotides long. Cas9 makes a "double-stranded break" in the DNA double helix by severing both strands using two different portions, or "domains," on its structure. Cas9 has a safety feature that stops it from randomly chopping a genome. Protospacer adjacent motifs, or PAMs, are brief DNA sequences that serve as tags next to the target DNA region. Cas9 will not cut if there is no PAM adjacent to the target DNA sequence [8].

CRISPR-CAS9 gene editing system

Some bacteria and archaea contain *CRISPR*- Clustered, Regularly Interspaced Short Palindromic Repeats. In the case of bacteria, this is the natural defensive mechanism. The DNA strand of invasive infections is cut and destroyed by the nuclease CAS9 protein. The *CRISPR* gene sequences were found in *E. coli* in 1980, but their function was unknown then [9].

CRISPR-CAS9 in genetic engineering

Only the dimeric CAS9 protein and guided RNA are required for gene editing. The double-stranded cut is produced once the nuclease cleaves the DNA at the intended location. The cell's natural DNA repair machinery currently uses non-homologous end-joining or homologous direct repair to fill the gap. Repairing double-stranded breaks is essential to the overall system's functionality [10].

Non-homologous end-joining

By joining two non-homologous strands that are immediately opposite one another, the gap is directly repaired in this type of DNA repair process [11]. The absence of the whole DNA fragment in NHEJ may result in the loss of several crucial genetic details. In the succeeding cell cycle, this DNA fragment will never be present, resulting in a mutation in the genome [12].

Working of *CRISPR*

Bacteria have the *CRISPR* pathway, which acts as a kind of defence system against plasmid DNA and viruses that are introduced into the organism [13]. The bacterial genome contains *CRISPR* loci that operate as "memory" of prior infections by incorporating brief DNA sequences (spacers) from invading viruses. When there is a re-infection, the complementary mature *CRISPR* RNA (crRNA) searches for a matching sequence, giving the *CRISPR*-associated (Cas) nuclease the specificity to generate a double-strand break at particular "foreign" DNA sequences [14].

For the development of such a tool, the Cas9 nuclease and a guide RNA (gRNA) were reduced to the essential elements of the endogenous *CRISPR* process. The crRNA and tracrRNA make up the two halves of the guide RNA. The crRNA has a brief homology region that allows it to attach to the tracrRNA and targets the double-stranded DNA to be cut. The Cas9 protein connects with the stem-loop shape that the tracrRNA offers. The term "gRNA" refers to the duplex of crRNA and tracrRNA. A Cas9 Ribonucleoprotein (RNP), which is made up of the Cas9 nuclease and gRNA, may attach to and cut a particular DNA target in the context of the entire genome. The RNP can only cleave targets that contain two particular sequences.

The protospacer-a set of 17-21 RNA-DNA homology bases-is the first requirement for the gRNA. The second need for the Cas9 protein's ability to connect to the target DNA is a brief Protospacer Adjacent Motif (PAM). If linking tracrRNA is present, and enough homology exists between the gRNA and the genomic target, the RNP cleaves both strands of the target DNA, creating a DSB at this precise location in the genome [15].

The components of *CRISPR* as a molecular tool can be delivered through a variety of techniques, despite the fact that the functional *CRISPR* in the nucleus is in RNP form. Early research was successful in producing a chimeric single guide RNA, or sgRNA, that replaces the duplex seen in nature with a single strand that incorporates the crRNA and tracrRNA. This sgRNA and Cas9 mRNA can be produced from a single plasmid and used for lentiviral transduction or direct transfection. Mix synthetically produced crRNA and tracrRNA with recombinant Cas9 protein to form an RNP that may be injected or transfected into embryos [16].

Following the creation of a targeted DSB, the cell normally uses either the Non-Homologous End Joining (NHEJ) or Homology-Dependent Repair (HDR) DNA repair pathways to continue functioning. These repair systems frequently produce errors, leading to mutagenesis at the target site, either by breaking a coding sequence to inactivate or knock out a gene (NHEJ) functionally or adding new DNA to knock in specific sequence alterations (HDR). Thus, the *CRISPR*/Cas9 system permits chromosomal DNA alterations that are irreversible and heritable. When Cas9 endonuclease domains are inactivated and coupled to other effector molecules to function at the gRNA-specified spot in the genome, the *CRISPR* system can also be employed as a targeted delivery mechanism [17].

This expands the *CRISPR* system functions to gene activation and inhibition [18]. Finally, the *CRISPR* system can be used for screening. All four primary uses of the *CRISPR*/Cas9 system gene knockout, gene knock-in, gene activation or inhibition, and screening are further discussed below.

Gene knockout

When *CRISPR*/Cas9 is co-expressed with a gRNA specific to the gene to be targeted, knockout cells or animals are produced. Gene knockouts alter a gene's expression in a cell to disclose the gene's function. A properly constructed, co-expressed gRNA instructs Cas9 to cleave a target sequence and create a DSB in the target gene [19]. Then, one of three methods can be used to produce gene knockout:

1. In order to repair the break, the cell uses NHEJ, which results

in random insertions or deletions (also known as "indels") within the cleaved gene's Open Reading Frame (ORF)

2. By inserting a specific disruptive sequence into the ORF using HDR and a user-supplied template, the cell fixes the break

3. Two DSBs produced by a pair of gRNAs border an important coding region, leading to its excision [20].

The most frequent repair method, NHEJ, introduces frameshifts and premature stop codons and/or targets the resultant transcript for Nonsense-Mediated Decay (NMD) since it is error-prone. An excessively short, nonfunctional protein can emerge from frameshift changes that put premature stop codons into the transcript and stop important portions of the amino acid chain from being translated. Removing mRNA transcripts with early stop codons and nonsense-mediated decay works to lower gene expression mistakes. Exon-exon junction complexes, which are protein complexes that form on the pre-mRNA strand between exons during RNA splicing, are thought to be responsible for activating this surveillance mechanism when the ribosome fails to remove them after a transcript has been spliced properly [21]. The transcript is signalled for destruction when an exon-exon junction complex is still bound as a result of an upstream frameshift, and the functional protein is never translated. Both of these processes are efficient at preventing a cell's genes from functioning normally.

Gene knock-in

New DNA sequences can also be "knocked in" using *CRISPR*/Cas9. Common changes include a Single Nucleotide Polymorphism (SNP), a tiny tag, loxP, or a bigger cassette like a fluorescent protein. These alterations are accomplished through a Cas9-induced DSB at a particular site, greatly enhancing the possibility for targeted integration. Through HDR, targeted integration (gene knock-in) takes place [22]. A DNA "donor" or repair template carrying the desired sequence, typically on a donor plasmid or oligonucleotide, must be given to the cell along with the gRNA and Cas9 in order to enable gene editing by HDR. The efficiency of gene knock-in is generally lower than for knockout (<10% of modified alleles) but can be used to generate specific modifications ranging from a single nucleotide change to large insertions [23-26].

Recent advances

1. Research Advances
2. Germline Editing
3. Agriculture
4. To Expedite Genetic Gain for Production Traits In Aquaculture
5. Biotic Stress Resistance
6. Abiotic Stress Resistance
7. Yield Improvement
8. Nourishment Enhancement

Ethical Concerns

1. When an embryo cannot consent to treatment, is it acceptable to utilise gene therapy on it? Is getting permission from the parents enough?

2. What if access to and affordability of gene therapies is restricted to wealthy individuals? If so, this could exacerbate already-existing health disparities between rich and poor.

3. Will some people employ genome editing for characteristics like athletic prowess or height that are not crucial for health? Is that all right?

4. Should germline cells ever be allowed to be edited by scientists? Generational changes to the germline would be transmitted.

Conclusion

Rapid development in the fields of medicine, genomics, aquaculture, agriculture and so many others due to advances in genome editing that enhances growth in the aquaculture sector, gene editing, diseases treatment etc. With fewer off-target consequences, *CRISPR*/Cas technologies have further improved functional capabilities. Additionally, the process of developing better gene modification tools is advancing and may eventually replace even *CRISPR*/Cas, transitioning to synthetic genomics.

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