

Crispr-Cas9 Revolutionizing the Blueprint of Life

Shivani Das

College of Veterinary Science & Animal Husbandry
Odisha University of Agriculture & Technology
Bhubaneswar, Odisha, India

Abstract

A revolutionary gene-editing technology, the CRISPR-Cas9, is capable of making precise modifications of DNA in many organisms. Originating from a bacterial defense mechanism, It makes use of the Cas9 enzyme to produce double-strand breaks and a guide RNA to target particular DNA sequences. It permits the replacement, deletion, or insertion of the genetic material and is used in genetic research, agriculture and medicine. Much attention has been drawn to the technology's accessibility and efficiency, both of which have inspired ethical debates over how it might be applied, mainly animal and human germline editing. Overall, CRISPR-Cas9 represents a transformative tool in biotechnology with intense implications for science and society.

Introduction

CRISPR-Cas9 which is also referred to as revolutionary gene-editing tool, has revolutionized the fields of genetics and molecular biology. Adopted from the immune system of bacteria, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its associated protein Cas9 offer an opportunity for these microorganisms to fight viral diseases. A guide RNA (gRNA) designed to correspond to a specific DNA sequence in the target genome is employed in the functioning of the technology. When gRNA is injected into a cell, it directs Cas9 enzyme to the correct DNA site and Cas9 then results in a double-strand break. The cell then repairs this break naturally, and they are employed in adding, deleting, or rewriting genetic information. CRISPR-Cas9 is easy to use and highly accurate, which is why researchers appreciate it so much. It has also facilitated major progress in functional genomics, crop improvement,

and potential treatment strategies for patients with genetic diseases. However, the technology also creates an ethical concern, especially in germline editing for human beings, thus there is always debate on the editing of the human gene. CRISPR-Cas9 is overall the highest biotechnological innovation and will provide powerful future capabilities for genetic research and therapy.

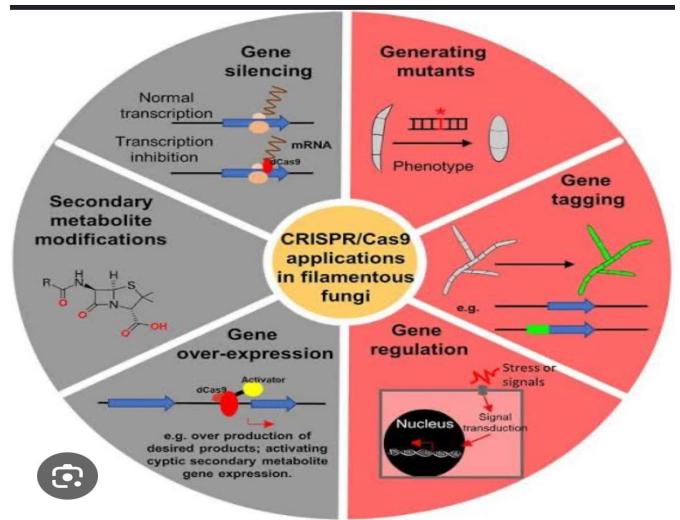
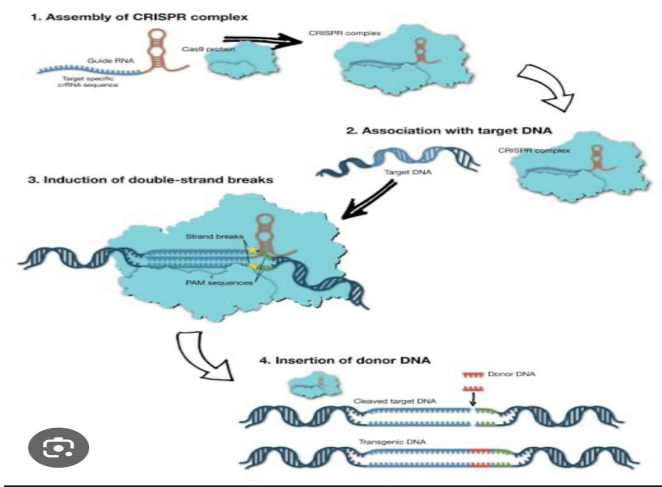
Mechanism :

It consists of 3 principal steps –

- ❖ Spacer Acquisition
- ❖ crRNA Processing
- ❖ Interference

Spacer Acquisition :

Spacer acquisition is a critical step in the CRISPR-Cas9 immune response and enables bacteria to adapt to and respond to viral infections.



Bacteria can then use this process to incorporate short pieces of viral DNA (called spacers) into their CRISPR loci, which can subsequently be used to identify and attack the same viruses upon subsequent infections.

Mechanism of Spacer Acquisition –

- 1. Recognition of Foreign DNA:** If a virus (bacteriophage) infects a bacterium, this foreign DNA is recognized by the bacterial immune system as foreign.
- 2. Integration of Spacers:** The CRISPR-associated (Cas) proteins help to incorporate a short portion of viral DNA (about 20 to 40 base pairs) into the CRISPR array within the bacterial genome. This new segment becomes a "spacer" and is inserted between repeating sequences (the repeats) in the CRISPR locus.
- 3. Cas Proteins Involvement:** The integration as well as the acquisition of these spacers is performed by specific Cas proteins, namely Cas1 and Cas2. Together they create a complex that recognizes, digests, and incorporates the viral DNA into the CRISPR array.
- 4. Adaptive Immunity:** Once the spacer is incorporated, it serves as a genetic memory of the viral infection. If the bacterium is infected again by the same or a similar virus, the CRISPR system can produce a corresponding RNA transcript from the spacer sequence.
- 5. Transcription and Processing:** The transcribed RNA is processed into a guide RNA (gRNA), which, together with Cas9, can target and cleave the viral DNA during a

subsequent infection, thus providing the bacterium with adaptive immunity against that specific virus.

crRNA Processing :

crRNA processing in the CRISPR-Cas9 system is a vital step that converts the genetic information stored in the CRISPR array into functional RNA molecules that guide the Cas9 enzyme to its target DNA. The crRNA processing steps are -

1. Transcription of the CRISPR Array :

The CRISPR locus, which contains repeated sequences interspersed with spacers (the viral DNA sequences), is transcribed to produce a lengthy precursor RNA (pre-crRNA). This pre-crRNA includes both the repeat and spacer sequences.

2. Formation of crRNA :

The pre-crRNA is processed to generate individual CRISPR RNAs (crRNAs). This processing is typically facilitated by Cas proteins, such as Cas9 and the processing enzyme Cas6.

The processing involves the cleavage of the pre-crRNA at specific sites, which are often located within the repeat sequences. This results in the release of individual crRNAs, each corresponding to a specific spacer sequence.

3. Integration with Trans-activating RNA (tracrRNA):

In many CRISPR systems, particularly Type II systems like CRISPR-Cas9, a trans-activating RNA (tracrRNA) is also involved. The

tracrRNA is complementary to the repeat sequences of the crRNA and helps stabilize and guide the crRNA. The tracrRNA and crRNA can form a complex, which is necessary for the effective binding of Cas9.

4. Formation of the gRNA :

In engineered CRISPR systems, the crRNA as well as tracrRNA may be fused into a single gRNA. This simplification allows for easier design and use in gene editing applications. The gRNA retains the ability to guide Cas9 to the target DNA sequence.

5. Complex Formation with Cas9 :

The processed gRNA (or crRNA-tracrRNA complex) binds to the Cas9 protein, forming the active ribonucleoprotein complex. At this point, the complex can identify and attach itself to the genome's corresponding target DNA sequence.

Interference :

Interference in the CRISPR-Cas9 system refers to the process by which the system recognizes and cleaves foreign DNA, particularly from viruses (bacteriophages), thus providing immunity to the host bacteria. This step is crucial for the adaptive immune function of CRISPR-Cas systems. The steps of interference are :

1. Recognition of Foreign DNA :

When a virus infects a bacterium, the CRISPR-Cas system detects the DNA of the virus as foreign. The previously acquired spacers (short segments of viral DNA stored in the CRISPR array) are key to this recognition.

2. Transcription of crRNA :

The spacers and repeat sequences seen in pre-crRNA, the precursor RNA produced during transcription of the CRISPR locus. After that, this pre-crRNA is processed into distinct CRISPR RNAs (crRNAs).

3. Formation of the gRNA-Cas9 Complex :

The ribonucleoprotein complex is formed when the processed crRNA attaches to the Cas9 protein. Currently, this complex can recognize and cut complementary DNA sequences.

4. Target Binding and PAM Recognition :

The gRNA-Cas9 complex scans the bacterial genome and any invading viral DNA for sequences that match the crRNA. For Cas9 to attach to the target DNA, a protospacer adjacent motif (PAM) must be present.

5. DNA Cleavage :

Cas9 causes a double-strand break in the viral DNA when it comes across a target sequence that is complementary to a nearby PAM. This cleavage effectively neutralizes the threat posed by the virus.

Applications of CRISPR-Cas9 Technology :

Applications for CRISPR-Cas9 technology are numerous and span several industries, including fundamental science, agriculture, and medicine. Some important applications:

1. Gene Editing

Knockout and Knock-in Studies: CRISPR-Cas9 may be utilized to disrupt (knockout) or insert (knock-in) specific genes to study their function and role in biological processes.

2. Disease Modeling

Cell and Animal Models: Researchers use models of human diseases (e.g. cancer, genetic disorders) to edit genes in cell lines or animal models to help find out more about disease mechanisms and potential treatments.

3. Therapeutic Application

Genetic Disorders: CRISPR-Cas9 can correct abnormalities at the DNA level, and so could be used to cure hereditary diseases such as muscular dystrophy, sickle cell anemia, and cystic fibrosis

Cancer Therapy: They are exploring the technology as a way to target, and perhaps modify, cancer cells to make existing therapies more effective or to develop new ones.

4. Agricultural Improvements

Crop Engineering: CRISPR-Cas9 is used to make genetically engineered crops with the desired

characteristics such as disease and drought resistance, and increased nutritional value.

Livestock Modification: The technique can be applied to enhance traits in livestock, such as disease resistance and growth rates.

5. Gene Drives

Population Control: Gene drives that spread specific genetic changes through populations might be advanced by CRISPR-Cas9, for example, for pest species or disease vector control, such as mosquitoes.

6. Synthetic Biology

Metabolic Engineering: CRISPR-Cas9 can be used by researchers to engineer microbial strains to produce biofuels, pharmaceuticals, and other valuable compounds.

7. Diagnostics

Pathogen Detection: Rapid and accurate detection of specific pathogens, including viruses and bacteria, is being developed using CRISPR-based diagnostic tools.

8. Ethical and Regulatory Discussions

Human Germline Editing: While technically feasible, editing human embryos raises significant ethical concerns and has sparked debates about the implications for future generations.

9. Biotechnological Research

Functional Genomics: CRISPR-Cas9 enables systematic gene knockouts to understand gene function and is used today in much of fundamental biological research.

10. Pharmaceutical Development

Target Identification: By systematically disrupting genes, and observing the resulting phenotypes, CRISPR can be used to identify new drug targets.

CRISPR-Cas9 is a versatile technique that has transformed the landscape of genetic research and biotechnology. This technology has great promise in solving complex problems in medicine, agriculture, and other areas while generating important ethical and regulatory considerations that must be addressed as this technology evolves.

References :

1. <https://en.wikipedia.org/wiki/CRISPR>
2. <https://www.yourgenome.org/theme/what-is-crispr-cas9/>
3. <https://www.nature.com/articles/nprot.2013.143>
4. <https://pmc.ncbi.nlm.nih.gov/articles/PMC4975809/>
5. <https://medlineplus.gov/genetics/understanding/genomicresearch/genomeediting/>
6. <https://www.genome.gov/genetics-glossary/CRISPR>