

CRISPR

[Clustered Regularly Interspaced Short Palindromic Repeats]

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Abstract

CRISPR is a groundbreaking tool in genetic engineering and molecular biology that edits and manipulates organisms' DNA with great accuracy. They function as a type of genetic memory, helping cells to detect and eradicate viruses more accurately. The CRISPR process consists of a sequence of stages that enable precise DNA editing in live organisms. To protect against viral infections, bacteria and archaea have spontaneously evolved an adaptive immune response mechanism. The capacity to change genes in bacteria, plants, animals, and even human cells with CRISPR brings up new possibilities for biotechnology, agriculture, and medicine.

Introduction

CRISPR is a kind of short palindromic repeating DNA sequences that are broken up by genetic code sequences known as spacer elements or spacers. It is a revolutionary technology in the field of genetic engineering and molecular biology. It edits and manipulate the DNA of organisms with high precision. The innate defensive systems of bacteria and archaea, a class of single-celled microorganisms were the model for CRISPR technology. They operate as a kind of genetic memory, allowing the cells to recognize and eliminate infections with more accuracy. This is especially true for viruses called bacteriophages. These species defend against viral and other foreign body assaults by using CRISPR-derived RNA and other Cas proteins, such as Cas9.

History: Key Events

The history of CRISPR spans several decades, with key developments in understanding the system and its potential applications occurring over time. The major milestones in the history of CRISPR:

1. **Discovery of CRISPR Sequences (1987-2002):** The first mentions of CRISPR sequences in 1987 when scientists noticed unusual repeats in the DNA of bacteria and archaea. In the



- following years, researchers identified these clustered regularly interspaced short palindromic repeats (CRISPR) in the genomes of various microorganisms.
2. **Hypothesis of Adaptive Immune System (2005):** In 2005, researchers proposed that CRISPR sequences could be part of an adaptive immune system in bacteria and archaea, providing protection against viral infections.
 3. **Identification of Cas Proteins (2005-2007):** Subsequent studies identified and characterized the Cas (CRISPR-associated) proteins associated with CRISPR sequences. Cas proteins were found to play a role in the defence mechanisms of bacteria and archaea against viruses.
 4. **Demonstration of CRISPR Function (2008):** In 2008, researchers demonstrated that CRISPR systems in bacteria could indeed target and cleave specific viral DNA sequences.
 5. **Development of CRISPR as a Genome Editing Tool (2012):** The breakthrough for using CRISPR as a powerful genome editing tool came in 2012. Jennifer Doudna and Emmanuelle Charpentier found the development of a simplified two-component CRISPR system using the Cas9 protein. This system allowed for precise and efficient gene editing in a wide range of organisms.
 6. **Rapid Adoption and Expansion (2013-Present):** The scientific community quickly adopted the CRISPR-Cas9 system for a range of applications, involving gene editing, gene control, and functional genomics, due to its convenience of use and adaptability. The genomes of microbes, plants, animals, and even cells of people have all had their genomes altered because to this technique.
 7. **2020 Chemistry Nobel Prize Winners:** Jennifer Doudna and Emmanuelle Charpentier acquired the 2020 Chemistry Nobel Prize for their ground-breaking work inventing the CRISPR-Cas9 gene-editing mechanism.

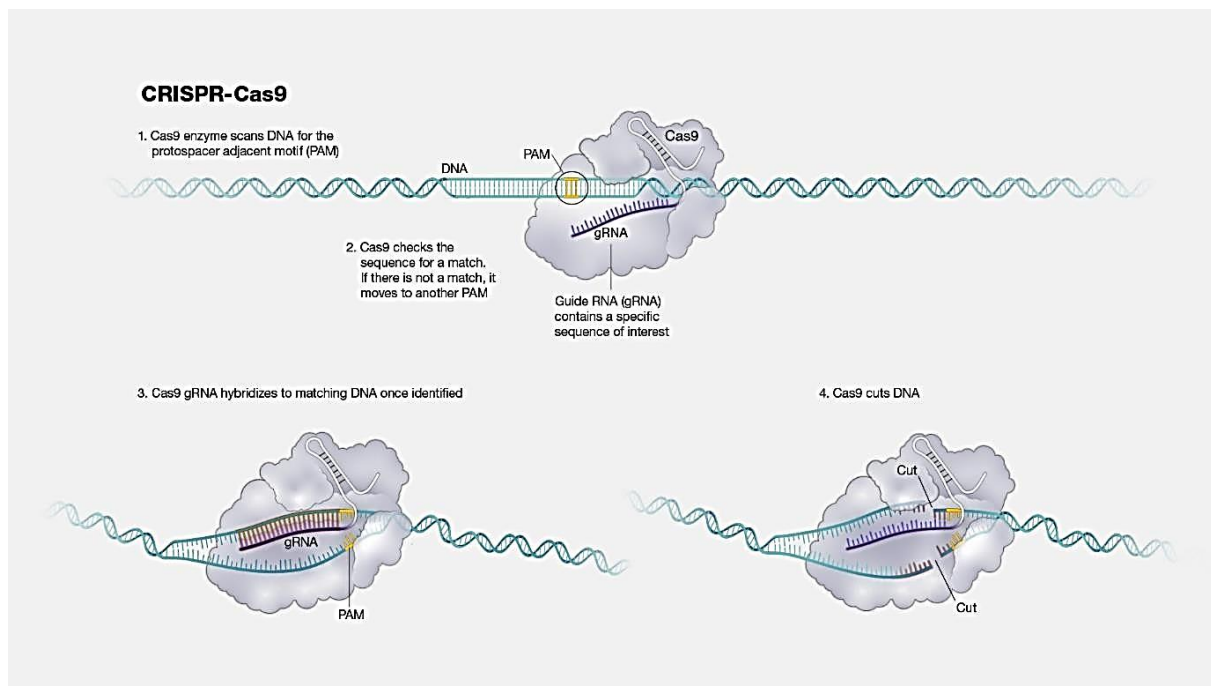
The discovery and development of CRISPR technology have revolutionized the field of genetic engineering and molecular biology. Its ease of use, precision, and versatility have made it a transformative tool with applications ranging from basic research to potential therapeutic interventions for genetic disorders. The ongoing research in CRISPR continues to refine and expand its capabilities, raising both excitement and ethical considerations about its use in various fields.

CRISPR mechanism:

The CRISPR mechanism involves a series of steps that allow for the precise editing of DNA in living organisms. The system has naturally evolved as an adaptive immune response in bacteria and archaea to defend against viral infections. The CRISPR system include CRISPR arrays, guide RNA (gRNA), and Cas proteins, with Cas9 being the most commonly used in



genome editing. These are the following steps of mechanism:



1. Acquisition of Spacer Sequences:

- Bacteria and archaea encounter viruses and other foreign DNA.
- The organisms integrate short fragments of the foreign DNA (spacer sequences) into their own genomic DNA at the CRISPR locus.

2. Transcription and Processing:

- The transcription of the CRISPR locus results in a lengthy precursor RNA molecule.
- This RNA is subsequently processed into smaller CRISPR RNA molecules (crRNA) that include the spacer sequences.

3. Formation of CRISPR-Cas Complex:

- The crRNA combines with Cas proteins to form a CRISPR-Cas complex.
- The complex is guided by the crRNA to recognize and bind to complementary DNA sequences, especially those derived from viruses encountered earlier.

4. Target DNA Recognition:

- The CRISPR-Cas complex surveys the genomic DNA of the organism, looking for sequences that match the crRNA.
- Through base-pairing between the corresponding DNA sequence and the crRNA, the assembly recognizes the target DNA sequence.

5. DNA Cleavage:

- Once the target DNA sequence is recognized, the Cas protein (e.g., Cas9) induces a double-strand break in the DNA at the specific location.
- This break activates the cell's DNA repair machinery.



6. DNA Repair:

- The cell repairs the DNA break through one of two main repair pathways:
 - Non-Homologous End Joining (NHEJ): This pathway often introduces small insertions or deletions (indels) during the repair process, disrupting the original DNA sequence.
 - Homology-Directed Repair (HDR): This pathway can be harnessed for precise gene editing by providing an exogenous DNA template. The cell uses this template to repair the break, incorporating the desired changes.

7. Editing the Genetic Material:

- Scientists can employ CRISPR to create targeted modifications in the genome by supplying template DNA for the repair process.
- This makes it possible to add, elimination, or exchange out certain DNA sequences.

By manipulating the guide RNA sequence and the Cas protein, now days scientists can reprogram the CRISPR system to target specific genes in a wide range of organisms. This adaptability and precision make CRISPR a powerful tool for genetic engineering and genome editing. The ability to alter genes in bacteria, plants, animals, and even human cells through the use of CRISPR opens up new avenues for biotechnology, agriculture, and medical applications.

CRISPR technology has immense potential in various applications, including:

1. Gene Editing: Researchers may examine the function of genes and possibly create cures for genetic illnesses by using CRISPR to precisely tweak particular genes.
2. Disease Treatment: CRISPR holds promise for treating genetic diseases by correcting or replacing faulty genes.
3. Agriculture: CRISPR can be used to enhance crop resistance to diseases, improve nutritional content, and develop other desirable traits in plants.
4. Biotechnology: CRISPR is widely used in biotechnology for a variety of applications, including the production of biofuels and pharmaceuticals.

The application of CRISPR technology, although extremely promising, also presents ethical questions since it may alter DNA sequences in embryonic stem cell lines, potentially leading to germ-line (sperm and egg) genome alteration in humans. The birth of these "designer infants" spurred discussions regarding the ethics of granting some people access to the technology and the morality of interfering with human development. In late 2018, twin girls in China were the first living beings to be genetically altered; their altered genes diminished the likelihood of contracting to HIV infection. There are ongoing discussions and



debates about the responsible use of CRISPR technology, and regulations are being developed to address these ethical considerations.

Conclusion:

Finally, CRISPR technology is a game changer in genetic engineering, with its capacity to accurately modify DNA opening up previously unheard-of possibilities in a variety of sectors. In medicine, CRISPR has the potential to treat genetic diseases, malignancies, and viral infections. In agriculture, it promises to improve crop resilience and nutritional value, hence solving global food security concerns. However, the fast advancement of CRISPR technology needs serious ethical considerations and strong regulatory frameworks to address possible dangers including off-target impacts and unforeseen consequences. As research advances, prudent development and deployment of CRISPR will be critical to realizing its full potential to benefit society while reducing ethical and safety risks.

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