CRISPR/Cas9

UNDERSTANDING THE GENE EDITING TECHNIQUE AND ITS DIVERSE DEPLOYMENTS

Gene editing is a rapidly evolving field that has revolutionized the way we think about treating diseases and understanding genetic disorders. One of the most significant breakthroughs in this field is the CRISPR/Cas9 gene editing technique. CRISPR/Cas9 is a versatile and efficient gene editing tool that allows researchers to make precise, targeted modifications to DNA sequences in living cells. This article delves into the origin and history of CRISPR/Cas9, its molecular mechanism, the different types of Cas9, and its diverse deployments spanning multiple industries.


EMMANUELLE CHARPENTIER

ORIGIN AND HISTORY OF CRISPR/CAS9

The CRISPR system was first discovered in 1987 in E. coli, but its significance was not understood until 2005. In 2012, Emmanuelle Charpentier and Jennifer Doudna proposed that CRISPR could be used as a gene editing tool, describing how the system could be used to cut DNA. By the end of that year, several scientific groups had demonstrated that CRISPR/Cas9 could be used in mammalian cells. Since then, CRISPR/Cas9 has become one of the most widely used gene editing tools in research labs worldwide. (1,3)

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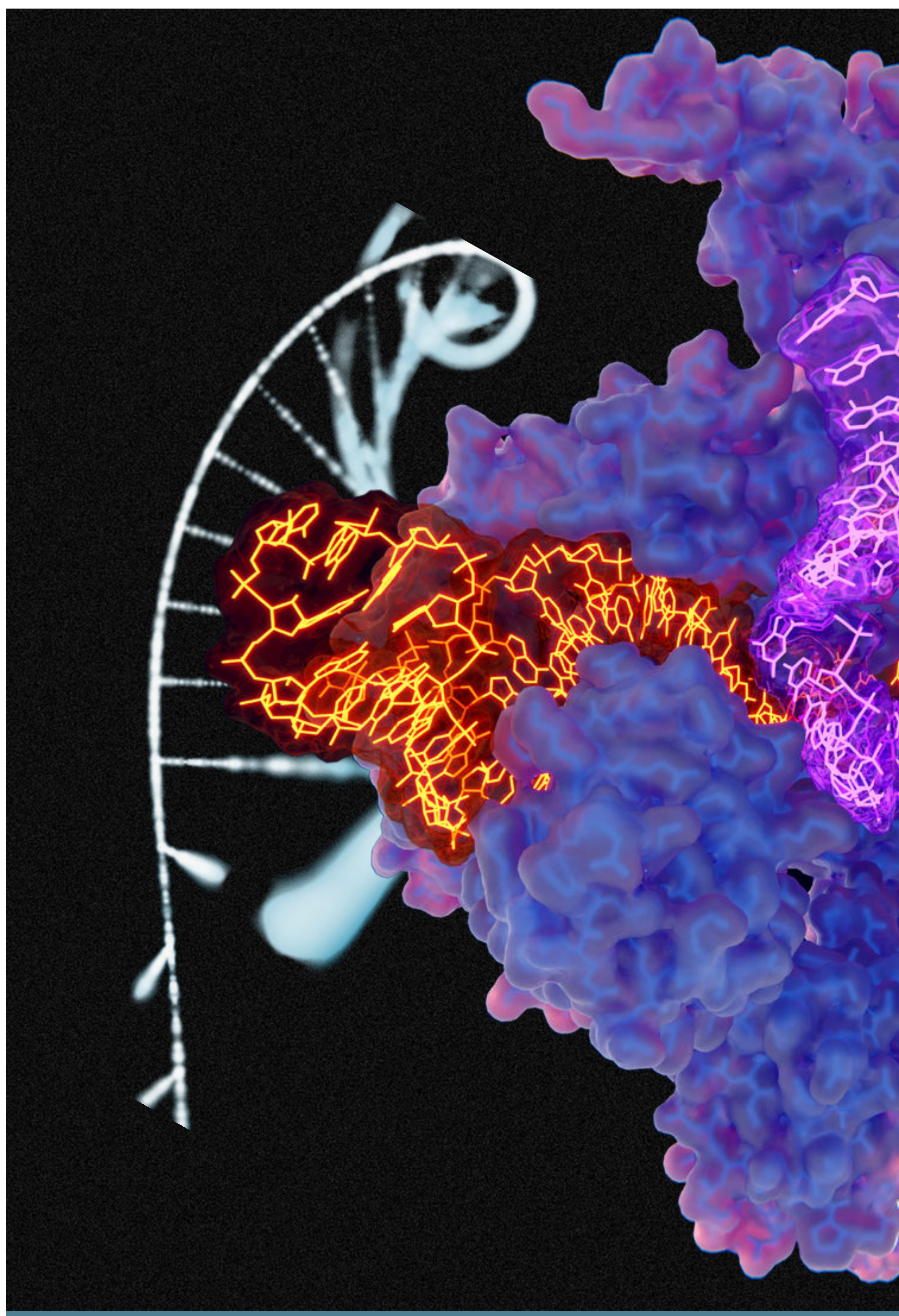
MOLECULAR MECHANISM OF CRISPR/CAS9

The molecular mechanism of CRISPR/Cas9 is a complex process that involves several steps. It all starts with the design of a guide RNA (gRNA), which is a synthetic RNA molecule that is engineered to match a specific DNA sequence in the genome. The gRNA consists of two components: a targeting sequence that matches the DNA sequence of interest and a scaffold sequence that allows it to interact with the Cas9 protein.

The Cas9 protein is an RNA-guided endonuclease enzyme that cleaves DNA at a specific location. Cas9 is derived from the bacterial immune system, where it functions to defend against invading viruses. Cas9 is composed of two main domains: the recognition domain, which interacts with the gRNA, and the nuclease domain, which cleaves the DNA

When the gRNA binds to the Cas9 protein, it forms a complex that can recognize and bind to a specific sequence of DNA. The gRNA serves as a guide, directing the Cas9 protein to the precise location in the genome where the DNA is to be cut. Once the Cas9 protein is bound to the DNA, it cleaves the DNA, creating a double-strand break.



After the CRISPR/Cas9 system creates a double-strand break in the DNA at a specific location, the cell's repair mechanisms come into play. The two main repair pathways are NHEJ and HDR.

Non-homologous end-joining (NHEJ) is a process in which the broken ends of the DNA molecule are reconnected, often with some nucleotides lost or added at the site of the break. This repair mechanism is fast and efficient but is also error-prone. As a result, NHEJ can introduce small insertions or deletions (indels) in the DNA sequence, causing a gene to lose its function or disrupt its normal activity. Indels can also affect regulatory regions, leading to changes in gene expression levels. (3)

Homology-directed repair (HDR) is a more precise repair mechanism that uses a homologous DNA template to guide the repair process. HDR occurs only during the S and G2 phases of the cell cycle when the cell has access to a homologous chromosome or a synthetic DNA template that is similar to the broken DNA sequence. (3)

The synthetic template is used to repair the double-strand break in a precise manner, such as by introducing specific nucleotide changes or by replacing a gene with a modified version. HDR can be used to insert large fragments of DNA or to make more extensive changes to the genome. However, HDR is a less efficient process than NHEJ and requires specific conditions to occur, making it more challenging to use in practice.

Overall, the choice of repair pathway depends on the cell type, the stage of the cell cycle, and the experimental conditions used. NHEJ is often preferred for gene knockout experiments, where the goal is to disrupt the function of a gene, while HDR is more suitable for precise editing of the genome, such as gene correction or gene replacement. (3)

CAS9 AND HOW IT HAS EVOLVED

As mentioned, there are several different types of Cas9 enzymes and each of them has its unique properties that make them suitable for different applications. The most widely used Cas9 enzyme is the one derived from Streptococcus pyogenes (SpCas9), which has been extensively characterized and optimized for gene editing. Other Cas9 enzymes have been identified from other bacterial species, such as Neisseria meningitidis, Staphylococcus aureus, and Francisella novicida, which have different properties and characteristics.

- **SaCas9:** derived from Staphylococcus aureus, is smaller than SpCas9 and can be more easily delivered to cells, making it a popular choice for in vivo applications.

- **FnCas9:** derived from Francisella novicida, is also smaller than SpCas9 and SaCas9 and has been shown to be highly specific in targeting DNA.

- **NmCas9:** derived from Neisseria meningitidis, is smaller than SpCas9 and has been shown to have high efficiency in DNA cutting.

In addition to these naturally occurring Cas9 enzymes, scientists have developed several modified versions of the Cas9 enzyme:

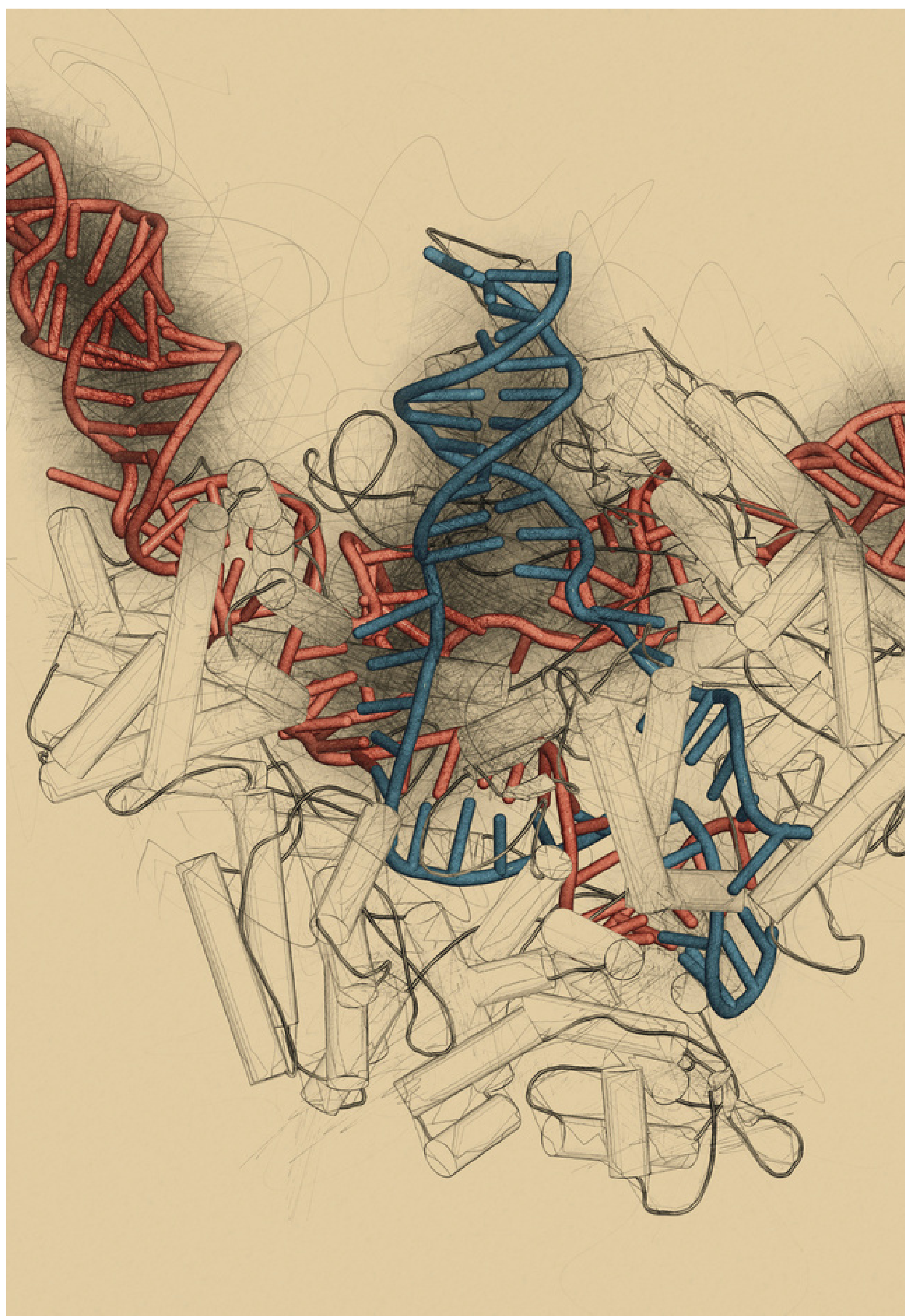
- **dCas9**: or "dead Cas9" is an enzyme that cannot cut DNA but can still bind to target DNA sequences, making it useful for gene regulation and transcriptional control.

- **Enhanced SpCas9**: an enhanced version of SpCas9 that has been engineered to have higher efficiency in DNA cutting and lower off-target effects.

- **HiFi Cas9**: a high-fidelity version of SpCas9 that has been engineered to have lower off-target effects and higher specificity in targeting DNA.

- **nCas9**: or "nickase Cas9" is an enzyme that has been mutated to cut only one strand of DNA, allowing for more precise editing with fewer off-target effects.

- **Cas9-X:** a Cas9 enzyme that has been engineered to recognize different types of DNA sequences, such as methylated DNA or RNA, allowing for more versatile genome editing applications.



APPLICATIONS

CRISPR/Cas9 has been used in multiple industries, including biotechnology, medicine, agriculture, and environmental science. Some of the examples of use cases include:

Biotechnology and Medicine

- Correcting genetic mutations that cause diseases such as sickle cell anemia and cystic fibrosis
- Creating new disease models by introducing specific genetic mutations into animal models, such as mice and zebrafish, that mimic the genetic mutations found in human diseases
- Developing new treatments for diseases by studying the underlying mechanisms of genetic mutations

Agriculture

- Improving crop yields and enhancing their resistance to environmental stressors such as drought, pests, and diseases
- Developing disease-resistant livestock with improved feed efficiency and meat quality

Energy

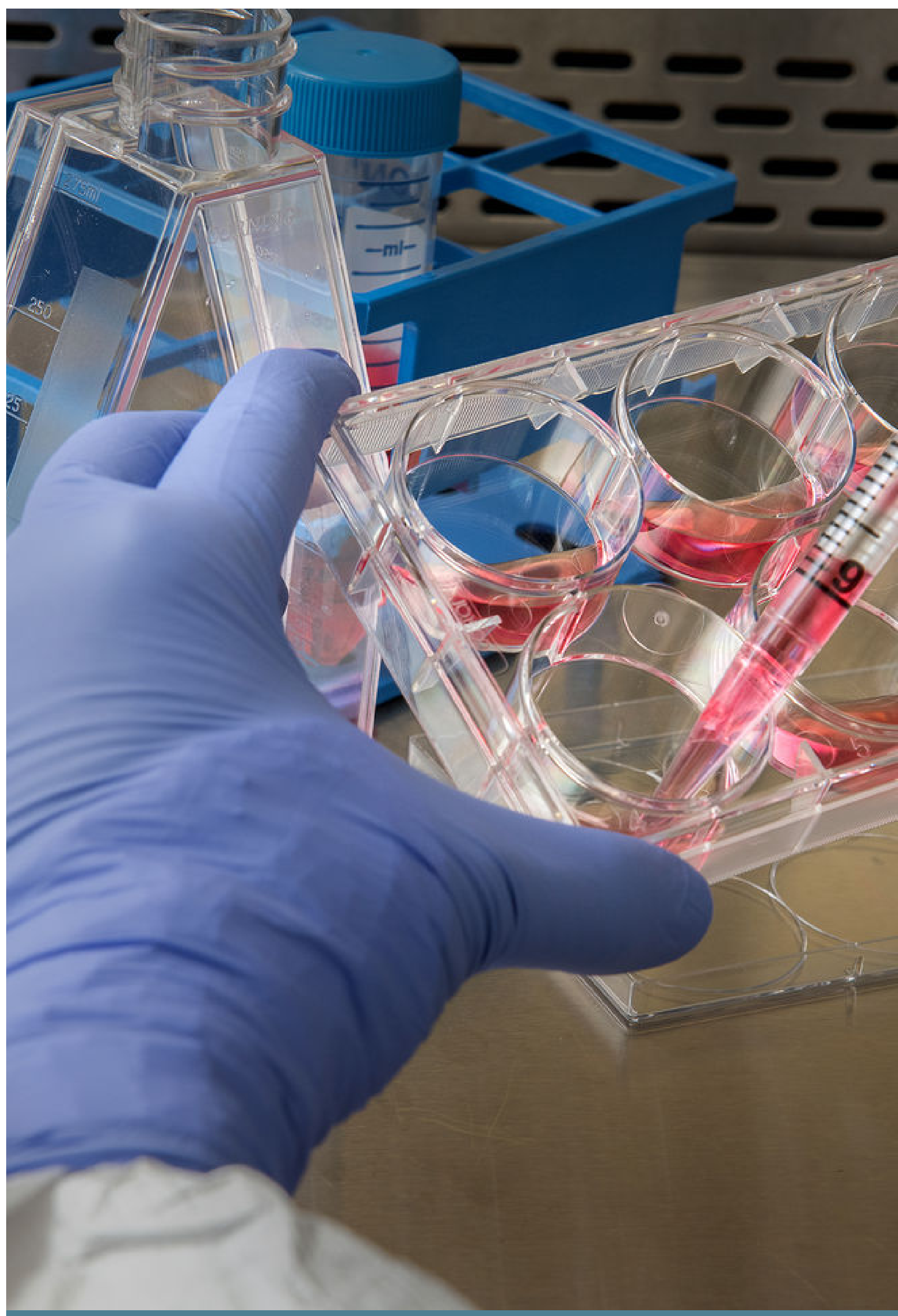
- Developing new sustainable technologies for biofuels production by engineering microorganisms that can convert biomass into useful fuels
- Enhancing the efficiency of photosynthesis in plants to increase biomass production and improve carbon fixation

Environmental Protection

- Developing new bioremediation strategies for cleaning up contaminated soils and water bodies by engineering microorganisms with enhanced pollutant degradation abilities
- Developing new biomaterials that are biodegradable and can replace traditional plastics

Basic Research

- Creating new genetic tools for studying gene expression and regulation
- Developing tools for genome editing that are more efficient and precise than current methods



CONCLUSION:

In conclusion, the CRISPR/Cas9 gene editing technique has revolutionized the field of genetic engineering, allowing researchers to make precise, targeted modifications to DNA sequences in living cells. The technique's origin and history can be traced back to the immune response of bacteria to viral infections, and the molecular mechanism of CRISPR/Cas9 involves several steps, including the design of a guide RNA and the use of different repair pathways.

The development of different types of Cas9 enzymes, each with its unique properties, has further expanded the range of applications of this technique across various industries. With ongoing advancements in this rapidly evolving field, CRISPR/Cas9 is set to continue transforming our understanding of the biological world around us, revolutionizing the ways we treat disease, farm, research and live.

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