



CRISPR-Cas Systems: Diversity, Mechanisms, and Applications in Biotechnology

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DESCRIPTION

CRISPR-Cas (Clustered regularly interspaced short palindromic repeats) systems, originally discovered as an adaptive immune mechanism in bacteria, have revolutionized the field of genetic engineering. This powerful technology, characterized by its simplicity, precision, and versatility, enables researchers to edit genomes with unprecedented accuracy. Understanding the diversity and mechanisms of CRISPR-Cas (Clustered regularly interspaced short palindromic repeats) systems is essential for appreciating their broad range of applications in biotechnology. CRISPR-Cas systems are classified into two main classes and six types, based on their structural and functional components. Class 1 systems, comprising types I, III, and IV, use multi-protein complexes for their interference mechanisms. Class 2 systems, including types II, V, and VI, utilize a single, multi-domain protein for interference. The most widely studied and utilized in biotechnology is the type II CRISPR-Cas9 system.

Type I CRISPR-Cas systems is prevalent in many bacteria and archaea, use a complex known as Cascade (CRISPR-associated complex for antiviral defense) to identify and degrade foreign DNA. Type III system targeting both DNA and RNA is providing robust defense mechanism. Type IV systems, though less understood, are thought to function similarly to type I systems but with distinct protein components. Type II systems, exemplified by CRISPR-Cas9, are the most studied due to their simplicity and efficiency. Cas9, a single endonuclease, is guided by a single-guide RNA (sgRNA) to create double-stranded breaks in target DNA. Type V and VI systems use proteins like Cpf1 (also known as Cas12) and C2c2 (Cas13), respectively, offer unique properties such as staggered DNA cuts and RNA targeting, expanding the toolkit for genome editing. The CRISPR-Cas mechanism involves three main stages: Adaptation, expression, and interference. During adaptation, fragments of foreign DNA, known as protospacers, are integrated into the CRISPR array in the host genome. These sequences serve as a molecular memory of previous infections. In the expression

stage, the CRISPR array is transcribed and processed into small CRISPR RNAs (crRNAs), each containing a unique spacer sequence derived from the protospacer. These crRNAs guide the Cas proteins to their corresponding target sequences in the invading genetic material.

The interference stage involves the recognition and cleavage of the target DNA or RNA by the Cas protein complex. In type II systems like CRISPR-Cas9, the Cas9 protein forms a complex with the sgRNA, which directs it to the complementary DNA sequence. The Cas9 protein then induces a double-stranded break at the target site, which can be repaired by the cell's natural repair mechanisms, leading to targeted gene modifications. The precision and versatility of CRISPR-Cas systems have made them indispensable tools in biotechnology, with applications spanning from basic research to therapeutic development. CRISPR-Cas9 enables precise modifications in the genome, allowing for the study of gene function, creation of Genetically Modified Organisms (GMOs), and correction of genetic mutations. This has profound implications for agriculture, where CRISPR is used to develop crops with improved yield, disease resistance, and nutritional value. CRISPR holds great promise for treating genetic disorders by correcting mutations at their source. Clinical trials are underway for diseases such as sickle cell anemia, cystic fibrosis, and muscular dystrophy. By delivering CRISPR components to affected cells, researchers aim to repair faulty genes and restore normal function. CRISPR is a powerful tool for dissecting gene function. By creating targeted knockouts or knock-ins, researchers can study the roles of specific genes in development, disease, and cellular processes. This accelerates the discovery of gene function and the identification of therapeutic targets.

CRISPR is used to engineer organisms with novel functions. By integrating new genetic circuits, scientists can create microbes that produce biofuels, pharmaceuticals, or biodegradable plastics. This has the potential to revolutionize industrial biotechnology and promote sustainable solutions. CRISPR

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enables the creation of precise animal models of human diseases, facilitating the study of disease mechanisms and the testing of new therapies. These models are invaluable for preclinical research and drug development. CRISPR-based diagnostics have ability to recognize specific nucleic acid sequences to detect

pathogens, genetic mutations, and biomarkers with high sensitivity and specificity. These tools have been particularly useful in the rapid detection of infectious diseases, including COVID-19.