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A Report on CRISPR-Cas System

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BRIEF REPORT

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a DNA sequence family found in the genomes of prokaryotic species like bacteria and archaea. These sequences are obtained from bacteriophage DNA pieces that infected the prokaryote before. During successive infections, they are employed to recognise and destroy DNA from related bacteriophages. As a result, these sequences play an important role in prokaryotes' antiviral (i.e. anti-phage) defensive mechanism and offer a sort of acquired immunity. CRISPR is present in around half of all sequenced bacterial genomes and virtually all sequenced archaeal genomes.

Cas9 (or "CRISPR-associated protein 9) is an enzyme that recognises and cleaves certain strands of DNA that are complementary to the CRISPR sequence using CRISPR sequences as a guide. Cas9 enzymes and CRISPR sequences constitute the foundation of the CRISPR-Cas9 technology, which may be used to alter genes within organisms. This editing method offers a wide range of applications, including fundamental biology research, biotechnological product creation, and illness therapy. The Nobel Prize in Chemistry was given to Emmanuelle Charpentier and Jennifer Doudna in 2020 for their creation of the CRISPR-Cas9 gene editing method.

The CRISPR/Cas system is a potent gene editing tool that allows scientists to precisely alter DNA sequences at any given position in the genome, significantly more precisely than traditional genome modification techniques. This system's discovery has ushered in a new era in molecular biology, with lucrative applications in medicine and industry. Because of its potential applicability in targeted genome and epigenome modification, CRISPR/Cas has become a fascinating topic of research in recent years, with the goal of uncovering the hidden science and facts behind complex life processes. Since the discovery of CRISPR/Cas in bacteria, researchers have been working hard to find newer and more effective CRISPR/Cas systems in order to expand the variety of applications in genetic engineering and molecular biology. CRISPR systems, which are found in 40% of bacteria and are practically universal in archaea, are adaptive immune systems that protect against invading foreign genetic material.

CRISPR-Cas systems have been classified into two classes and six primary varieties up to this point, with the CRISPR/Cas9 system garnering the most interest for genome engineering. The CRISPR-Cas9 system is based on the endonuclease protein Cas9's nucleolytic activity, which is steered to the target spot in the genome by a specificity determining RNA called guide RNA (gRNA). Apart from these, the CRISPR/Cas9 system recognises another sequence known as protospacer adjacent motif (PAM), which is located next to the target site and is critical for Cas9 activity. In the presence of gRNA, the Cas9 protein attaches to the target site with great precision and executes a double strand break followed by the insertion of indel mutations at the cleavage site via the cell's non-homologous end joining repair process. Specific nucleotides in the DNA can also be changed by homologous recombination mediated repair using a predesigned repair template. RuvC and HNH domains, respectively, are crucial residues important for Cas9's catalytic activity. A point mutation was used to replace both residues with Alanine, resulting in a nuclease-deficient Cas9 protein known as dead Cas9 or dCas9. Although dCas9's nuclease function was reduced, the protein was still able to precisely attach to DNA at a specific spot in the genome when directed by gRNA. The dCas system has expanded the range of applications for CRISPR-Cas9 technology beyond genome editing. Unlike genome engineering, the CRISPR/dCas system causes reversible gene expression regulation, and two independent methods have been created to do this. The process of transcriptional repression is referred to as CRISPR. Outside of gene editing, these techniques have been used extensively to modulate the expression of many genes and to determine their biological activities. This chapter provides an overview of gene expression regulation technologies, including their uses in biotechnology and medical research, as well as future advances.

To protect themselves from viral predators, prokaryotes have developed a number of defence mechanisms. CRISPR and its related proteins (Cas) show a prokaryotic adaptive immune

system that remembers prior infections by incorporating small stretches of invading genomes—dubbed spacers—into the CRISPR locus. The spacers between repeats are produced as short guide CRISPR RNAs (crRNAs), which Cas proteins use to target intruders sequence-specifically when an infection recurs. The capacity of the CRISPR-Cas9 system to target DNA regions with programmable RNAs has opened up new possibilities in genome editing in a wide range of cells and species, with significant therapeutic promise. While various scientific research have revealed information on the molecular pathways that underpin CRISPR-Cas systems, certain parts of the immunity phases remain unsolved. This overview reviews significant CRISPR-Cas findings, examines CRISPR-Cas' function in prokaryotic immunity and other physiological features, and explores the system's potential as a DNA editing method and antimicrobial agent.