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Research progress of CRISPR-Cas system application

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Abstract: **The CRISPR-Cas system is an adaptive immune defense system found in the genomes of 45% bacteria and most archaea in recent years, which can effectively resist the invasion of foreign genetic elements. In recent years, the CRISPR-Cas system has been used in many fields, such as gene editing, gene therapy, bacterial typing and evolution research.**

Keywords: CRISPR-Cas system; Application; Gene editing; Bacterial resistance

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1. Introduction

The CRISPR-Cas system is an adaptive immune defense system present in the genomes of bacteria and archaea, which can effectively resist the invasion of foreign genetic elements and maintain the stability of the bacterial genome[1]. Nowadays the CRISPR system is widely used in gene editing, bacterial typing research, gene diagnosis and disease treatment, energy and industry. Based on a brief description of the gene structure and mechanism of action of the CRISPR-Cas system, this article will review the related progress of its application research.

In 1987, Ishino Y of the research group of Osaka University in Japan discovered a repeating sequence when studying the nucleic acid sequence of the alkaline phosphatase isozyme Ipa in *E. coli* K12[1]. Until 2002, Jansen[1] conducted a careful study of the repeated nucleic acid sequences in the base sequence of this group of prokaryotes, and finally made a major discovery, named it CRISPR (clustered regularly interspaced short palindromic repeats). And the coding protein composed of conserved base sequences was named as cas protein (CRISPR-association proteins).

The CRISPR-Cas system consists of a leader sequence, a CRISPR array and a series of cas gene clusters, and the CRISPR array is composed of highly conserved repeat sequences (Direct Repeat) and spacer sequences, and they constitutes the R-S structure. The leader sequence is generally located upstream of the first repeat sequence, which is generally 100-500 bp in length and is rich in "A/T" sequences. Studies have shown that the leader sequence plays a crucial role in multiple stages of the role of the CRISPR-Cas system. In the vicinity of the CRISPR array, there are unequal numbers of genes encoding unknown proteins, called cas genes, which encode proteins containing nuclease polymerase, helicase and ribonucleic acid-binding domains, called Cas proteins. These Cas proteins can be divided into different types and subtypes of CRISPR systems, but they play an indispensable role in the process of CRISPR playing an immune defense mechanism.

The CRISPR-Cas system can be divided into two major categories according to the different genetic structure and mechanism of action, six types of I-VI, and it is further divided into 19 different subtypes[2,3] on this basis. When CRISPR-Cas plays an immune defense role, it can be divided into three stages：adaptation, expression and interference. When foreign genetic elements invade, CAS1 and CAS2 of the CRISPR-Cas system form a complex to take a prospacer from its genome and insert it between the leader sequence and the first repeat sequence to form a new spacer. When the same foreign genetic material invades again, the CRISPR can be transcribed pre-crRNA, and this molecule further binds to the CAS9 or multi-subunit complex to form a crRNA-effector complex to recognize and cleave DNA or homologous to the spacer or RNA, then completing an immune defense process (Figure 1).

2. Application of CRISPR-Cas system

2.1 Application of CRISPR-Cas system in the field of gene editing

The CRISPR-Cas9 system was first demonstrated in 2012 to specifically cleave double-stranded DNA and break it in vitro[4]. Mali et al. found that it can edit DNA in mammalian cells the following year[5]. CRISPR-Cas9 technology is widely used in gene editing research of animals and plants because of its high efficiency, simple structure and easy operation. Now CRISPR-Cas9 gene editing technology has been successfully used in zebrafish[6] mice, rats, pigs[7], rice, Arabidopsis[8] and other animals and plants, providing a powerful study of its gene function Technical support.

The CRISPR-Cas9 gene editing system is composed of Cas9 protein, CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA). This article

takes the Cas9 protein of Streptococcus pyogenes as an example to briefly introduce its mechanism of action. TracrRNA is a RNA with a hairpin structure formed by the transcription of a repeat sequence, which forms a complex with the precursor crRNA and Cas9 protein, and can promote the precursor crRNA to become a mature crRNA by activating RNaseIII, and then the mature crRNA binds to the target sequence and guides Cas9 to proceed Cut. Cas9 protein consists of two structures and structures, namely a REC domain with recognition function and a NUC domain with nuclease activity, and the NUC domain contains two highly conserved RuvC and HNH domains, which are the same as sgRNA for cleavage. And complementary single strand[9]. With the deepening of research, many new CRISPR-Cas gene editing systems have been discovered and modified, such as CRISPR/Cpf1, CRISPR/Cas12b, CRISPR/Cas13a, which has greatly expanded its application in the field of gene editing[6,10].

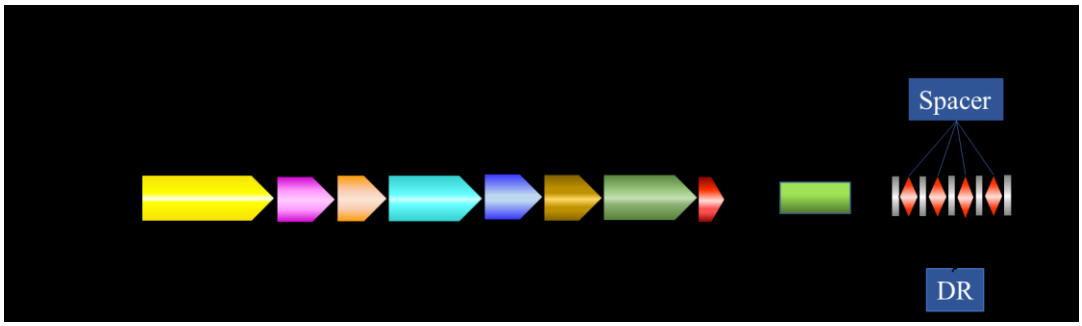

Figure 1. Schematic diagram of the gene structure of the CRISPR-Cas system.

In terms of gene function research, the current CRISPR-Cas9 gene editing technology has been successfully used to gene knockout and gene knock-in in the mouse, rat, Arabidopsis, pig and other animal models. In terms of mediating gene expression regulation, the transformation of CRISPR-Cas9 results in the formation of dCas9 with loss of nuclease activity but with DNA binding ability, which can be coupled with different proteins to up-regulate or down-regulate the expression of target genes. In terms of gene therapy, CRISPR/Cas9 can precisely edit the genome to achieve the purpose of treating diseases at the genetic level, the improved CRISPR/Cas9 has been successfully applied to single-base gene editing of DNA or RNA. For example, Liang et al.[11]used single-base gene editing technology to repair β-thalassemia patients HBB-28A Single-base mutations to G, the single-base editing efficiency in nuclear transfer human embryos can reach 23%. In addition, CRISPR/Cas9 is also used for immunotherapy of some tumors[12].

The discovery and application of CRISPR-Cas9 technology has greatly promoted the development of the field of gene editing. With further research, the technology will continue to improve and mature, which plays an important role in promoting the progress of life science research and human disease treatment.

2.2 Application of CRISPR-Cas system in bacterial typing and evolution

The classification and molecular typing of pathogens is an important issue in modern microbiology. The main purpose of microbial typing is to evaluate the relationship between microbial isolates to determine the source and route of infection, confirm or eliminate the epidemic, evaluate the cross-transmission of medical-related pathogens, and ultimately evaluate the feasibility of the control measures we want to take. Traditional typing methods include serotyping, phage typing and antimicrobial spectrum typing. In recent years, high-throughput and high-resolution molecular typing methods have been deeply integrated with epidemiological molecular studies, most of which are based on analysis of DNA sequence and PCR results.

As the main core gene of CRISPR analysis, the Cas gene is often associated with CRISPR arrays and becomes an important basis for CRISPR system typing. In the evolution of bacteria, with the invasion of foreign genetic elements, spacer sequences are continuously embedded and screened out, so CRISPR array has diversity. Based on it we can design appropriate primers according to the target gene, amplify the CRISPR array by PCR and analyze the sequencing results. We can analyze the composition of the spacer sequence and its relative position to classify the bacteria studied. We can analyze the affinity of the spacer sequence and its matching phage or plasmid, which can roughly infer the historical evolutionary relationship or origin of this CRISPR system .

Some studies have used CRISPR structural characteristics to type Campylobacter jejuni, and found the classic CRISPR array structure in most samples, and the typing results show that the

resolution is similar to traditional methods (AFLP and MLST)[13].The spacer sequence was found to be highly polymorphic in Salmonella, and a strong correlation was detected between the polymorphism in the array and serotype. In fact, the analysis of the newly introduced spacers is highly consistent with traditional serotyping, which indicates that the use of spacer sequences for typing can replace traditional serotyping. In this way, we can obtain more detailed typing results by analyzing the size and sequence of the CRISPR array in the bacterial genome. In addition, the diversity of spacer sequences can reflect the history of bacterial exposure to foreign DNA in different periods and different environments. Using this feature of the spacer sequence, you can study the evolution of bacteria and speculate on the genetic relationship between different bacteria.

2.3 Application of CRISPR-Cas system in the study of bacterial resistance

With the irrational use of antibacterial drugs and the development of new drugs, the resistance of bacteria has increased year by year, even to the point where there is no suitable antibiotic treatment. Therefore, it is particularly important to reveal the mechanism of bacterial resistance, explore the causes of bacterial resistance and find effective ways to eliminate resistance.

Studies have shown that the CRISPR-Cas system can resist the invasion of exogenous genetic material such as phages and plasmids, as well as the proliferation of multi-drug resistant plasmids. Inspired by the principle of the CRISPR system, the drug-resistant or virulence gene fragments are implanted into the CRISPR array to generate immune memory, and an appropriate carrier is selected to deliver it to the bacteria. It not only can specifically kill the pathogenic bacteria with the target fragment, but also can prevent the horizontal gene transfer of drug resistance or virulence genes at the same time. So as to achieve the purpose of treatment and treat people infected by pathogenic bacteria. With the success of the CRISPR-Cas system targeted resistance gene experiment, it has proved that this system has potential to become a sequence-specific antimicrobial agent[14,15]. Subsequently, in 2014, two articles described the use of CRISPR to target specific antibiotic-resistant bacteria. The researchers used genetic engineering to construct Cas9 nuclease, trans-activated crRNA (tracrRNA) and a series of plasmid-targeted or genome-targeted CRISPR RNA into phage vectors, and delivered them to bacteria to target antibiotic resistance genes. Kill pathogens or restore their susceptibility to antibiotics to treat multidrug-resistant infections without damaging normal flora[14,15].

2.4 Application of CRISPR-Cas system in industry, agriculture

In recent years, due to people's abuse of antibiotics, the resistance of foodborne pathogens has continuously increased. As far as the bacteria themselves are concerned, on the one hand, they can generate new drug resistance genotypes through mutations in their own genes, and on the other hand, they can increase drug resistance through the horizontal transfer of resistance genes. It promotes the increase of the number of multi-drug resistant strains, which makes the treatment of food-borne diseases more difficult. Nowadays the CRISPR-Cas system is commonly used in the classification of food-borne pathogenic bacteria, especially Salmonella, and it has been found that the above-mentioned classification method can be extended to the application of food-borne pathogenic bacteria outbreak investigation[16].

The strains used in the food processing industry require not only high use efficiency, but also food-originality, so the available strains are few and the efficiency does not meet the factory production requirements. The development of wild phage resistant strains in China was also based on the detection and analysis of CRISPR sequences, and the mode of action of CRISPR anti-phage completely reached the level of food safety, so its widely use in production is favorable[1]. Fermentation industry operators have always been most concerned about phage infection, because when the fermentation broth is intensified by phage infection, the normal fermentation cycle will be immediately destroyed, which not only extends the fermentation cycle but also reduces the quality of the product. Causes significant economic losses to operators or the state[1]. The CRISPR system can solve this problem by inserting spacer sequences homologous to known phage sequences into the CRISPR system of industrial bacteria. It can fundamentally improve the resistance of industrial bacteria to specific phages, thereby easily solving the problem of bacteriophage pollution in industrial production.

3. Conclusion

The CRISPR-Cas system is constantly innovating molecular biology in many ways, driving the development of many important and different genome projects. The CRISPR-Cas system also provides preliminary data for gene editing of mammalian genetic diseases, including human genetic diseases. In this brief review, we understand the basic structure of the CRISPR system and review its application areas. We are convinced that with the deepening of research and the continuous development of science and technology, the

application field of CRISPR-Cas system will continue to expand and play a more important role for life science research and disease treatment.

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