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Review Article

CRISPR and its Potential Scope in Molecular Biology and Medicine: An Appraisal

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Abstract

The focus of the present review is to shed light on the Clustered regularly interspaced short palindromic repeat which is known as CRISPR – Cas Technology. The CRISPR gene editing technique is one of the revolutionary aspects of editing gene which is a programmable ribonucleic acid (RNA). It is a nuclease guided detection procedure having target specific orientation in the genome. This simple and innovative approach replaces the cumbersome protein-based DNA editing expensive methods in use for instance transcription activator–like effector nucleases (TALENs) or zinc finger proteins (ZNFs). Since publications describing the applications for editing genome both in eukaryotic and prokaryotic cells, CRISPR Cas technology has flourished on a high scale across laboratories worldwide. The applications are potentially observed in the fields like therapeutics, personalized medicine, basic research, environmental research and agriculture. The limitations of CRISPR lies in the fact that there arises the chance of a point accepted mutation in the targeted strand and the chance of off targeted mutagenesis. It is a benchmark standalone gene editing tool that affords advantage as far as cleavage pattern and efficiency of gene editing is concerned.

Keywords: CRISPR- Cas 9 Technology; Gene Editing; RNA; TALENs; Zinc Finger Proteins

Introduction

In 2016 after long years of research in gene editing technique which got introduced for the first time in human trials. The approval from the National Institutes of Health (NIH) for the first application of CRISPR-Cas technology got initiated in a human trial [1,2]. This particular decision holds potential for the rare genetic disease treatment in future, e.g human immunodeficiency virus (HIV) infection, and through cell replacement in hematologic cell replacement. The CRISPR-Cas technology derives the adaptive immune system from prokaryotes which targets and cuts them off invading genetic elements from plasmids or phages [3-9]. The safety and efficacy any of the DNA editing tools are highly specificity dependent. In some of the studies it is found that the CRISPR-Cas may leads to unenviable off-target effects [10-12]. Due to this reason, several newer developments in methods of gene editing are constantly being worked upon in order to minimize and recognize off-target effects.

Milestones in CRISPR-Cas research

It was for the first time described in 1987 of the repetitive sequences which facilitated the interest in CRISPRs and their associated genes slowly increased throughout the 1990s and early 2000s [13]. Initially believed to participate in cellular DNA repair and replicon partitioning processes, first evidence that CRISPR-Cas systems display an adaptive prokaryotic immune system was delivered in 2005. Researchers were surprised as they found that most of the interspersed sequences interspaced between identical repeats derived from extra chromosomal DNA, more specifically from phage genomes and conjugative plasmids. The hypothesis was eventually proven two years later when scientists showed the incorporation of new spacers into a CRISPR-Cas locus of *Streptococcus thermophilus* after challenging the bacterium with a bacteriophage.

The newly acquired spacers always showed perfect complementarity to sequences on the phage genome and conveyed resis-

tance towards that particular phage upon a subsequent infection. Research interest of the CRISPR field soon accelerated, leading to new discoveries that helped to understand the basic mechanisms of the immune system. In 2008, the processing of the CRISPR transcript into mature crRNAs that guide the Cascade complex of the *E.coli* type I-E system was experimentally validated, also giving hints that DNA rather than RNA is targeted. The latter was confirmed in the same year as a study demonstrated that indeed DNA is the targeted molecule. This led scientists to think about the potential role that this prokaryotic immune system might play as a DNA manipulation tool.

Today, CRISPR-Cas9 is a frequently harnessed tool for genome editing purposes and major progress in understanding the underlying biochemical processes in RNA-guided Cas9 was presented in recent years. In 2010, researchers showed that Cas9 creates a single double-stranded break at a precise position on the target DNA. Further insight into the mechanism was delivered 1 year later as the involvement of another small RNA, called tracr RNA, was shown. The maturation of crRNA requires tracrRNA as well as Cas9 and RNase III. Evidence that the system would function heterologous in other bacteria was demonstrated in 2011, as the *S. thermophilus* type II CRISPR Cas system could provide immunity in *E. coli*. Other research had shown certain elements of the type II system, including the involvement of a PAM sequence in interference but the nature of the cleavage complex remained unknown.

In 2012, tracrRNA, which was previously known to be involved in crRNA maturation, was shown to also form an essential part of the DNA cleavage complex, with the dual tracrRNA: crRNA directing Cas9 to introduce double strand breaks in the target DNA. Further simplification of the programmed targeting was achieved by creating a single-guide RNA fusion of tracrRNA and crRNA that guides Cas9 for sequence-specific DNA cleavage. A few months following the description of the CRISPR-Cas9 technology, a number of publications demonstrated its power to edit genomes in eukaryotic cells and organisms, including human and mouse cells.

CRISPR-Cas is the only adaptive immune system in prokaryotes known so far. In this system, small guide RNAs (crRNA) is employed for sequence specific interference with invading nucleic acids. CRISPR-Cas comprises a genomic locus called CRISPR that harbours short repetitive elements (repeats) separated by unique sequences (spacers), which can originate from mobile genetic elements (MGEs) such as bacteriophages, transposons or plasmids. The so-called CRISPR array is preceded by an AT-rich leader sequence and is usually flanked by a set of Cas genes encoding the Cas proteins. To date, CRISPR-Cas systems can be divided into two main classes, which are further classified into six types and several subtypes. The classification is based on the occurrence of effector Cas proteins that convey immunity by cleaving foreign nucleic acids. In class 1 CRISPR-Cas systems (types I, III and IV), the effector module consists of a multi-protein complex whereas class 2 systems (types II, V and VI) use only one effector protein.

The CRISPR Locus and the Mechanism for CRISPR-Cas Technology

For adaptive immunity bacteria uses the CRISPR Cas technology. The locus of CRISPR comprises of a short palindromic repeat which are separated by short non repetitive sequences called spacers. Spacers initiate from invading DNA sources, for instance phages, which gets incorporated and copied into the locus of CRISPR whenever infection occurs. Close to this locus, a second locus encompasses a set of genes that encodes CRISPR-associated endonucleases (Cas) system, which incorporates cuts into the genome. When a repetitive infection permeates the same DNA, an RNA molecule (CRISPR RNA, or crRNA) forms a complex with Cas thereby transactivation of crRNA (tracrRNA) which in turn guides the nuclease to the exogenous sequence.

The Cas-RNA complex recognizes the target DNA which is complementary to the crRNA and which is adjoining to a specific 3-nucleotide locus (PAM, the protospacer-adjacent motif). The complex then cuts in order to deactivate the permeating DNA [14-17].

Various other nucleases are closely associated with CRISPR activity in different bacteria, mostly the topics in this review article refers to Streptococcus pyogenes, which is based upon the endonuclease Cas9. The CRISPR-Cas9 system involves two RNA molecules: crRNA, transcribed from the DNA spacers, and tracrRNA, the interaction with crRNA is a structure based requirement for inscribing of Cas9 (Figure 1). The major achievement of the study is, here the2 RNAs were hybridized in order to create single-guide RNA (sgRNA). This Cas9- sgRNA system demonstrated streamlined gene editing properties.

Anti-CRISPR mechanisms

A remarkable defence strategy is harboured by the prokaryotes in order to coexist with their viral predators. Despite the arms race between viral complementary parts and the bacteria phages develops strategies to subdue the various antiviral defence mechanisms.

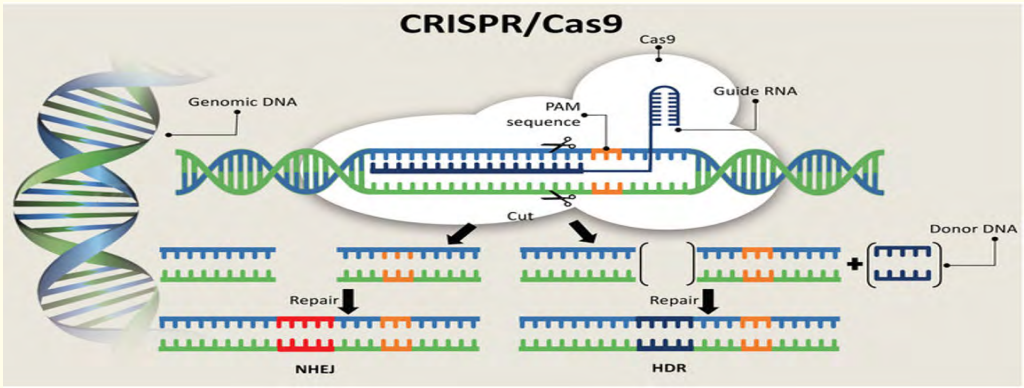

Figure 3: CRISPR- Cas 9 mechanism involves two distinct RNA molecules: crRNA, transcribed from the DNA spacers and tracrRNA a structure-based requirement for inscribing of Cas9.

The paragraph outlines the research on how CRISPR-Cas systems are evaded by phages. It is demonstrated from recent studies that Pseudomonas aeruginosa is infected by Mu-like phages, which inactivates their host's CRISPR-Cas systems. In turn anti-CRISPR (Acr) proteins are produced by these phages which interacts with constituents of the type I-F CRISPR-Cas interference mechanisms: e.g. AcrF1 and AcrF2 the phage proteins which binds at different subunits of cascade and thereby prevents the binding to target DNA of the Csy complex. The nuclease Cas3 where AcrF3 binds thereby impeding target degradation function. Type I-E CRISPR-Cas immunity is sometimes intercepted in the same organism, raising a question for other CRISPR-Cas types whether Acr proteins exists. Type I-FCRISPR-Cas system encoded by *Vibrio cholerae* ICP1 phages which target a host genome, involving CRISPR independent anti-phage defence. The defence mechanism of the host gets attacked thereby making it indispensable for phage propagation as the effectiveness of infection gets greatly lowered with deletion of targeting spacers in the viral CRISPR array. The analysis of phages shows that it can successfully infect the host acquired new spacers originating from the same genomic locus alongwith the virus perpetuating fully functional active CRISPR-Cas system [18,19].

Applications of crispr-cas9 Technology

CRISPR-Cas9 technology based gene editing is a major breakthrough in genomic research. Huge number of publications and research works pertaining to CRISPR association with "gene editing" is evidenced in the last few years. In CRISPR the significance of the number of studies addresses methodology and technical aspect, the multifaceted feature of these technology enabled its application into the biological systems [19].

The success and rapid adoption of CRISPR-Cas9 technology are, in part, due to the availability of next-generation sequencing (NGS). NGS can be used to detect target regions in the genome, validate accurate modification of the target, and detect any off-target effects. The expression and function of the gene can then be followed by next-generation RNA sequencing (RNA-Seq) and chromatin immune precipitation sequencing. These approaches can also be used to guide the optimization of the CRISPR-Cas9 system and to discover alternative gene editing systems [18,19].

The ability of the CRISPR-Cas9 system to insert or delete DNA sequences, simply and accurately, within living cells has been a long-held dream of researchers. This system allows for the deletion and insertion of genetic elements and syntenic regions to test the impact of these elements on a phenotype or disease. In different fields of medicine, the use of CRISPR-Cas technology in therapeutic approaches has become relevant progressively. The interspersed repetitive presence short spacers which is later known as CRISPR, has been utilized for the diagnostic reasons for simple typing of *Mycobacterium tuberculosis* strains.

This helped to understand ways used by pathogens for their transmission by looking at differences in the spacer content of related strains. This so-called spoligotyping (spacer oligotyping) has also been adapted for *Salmonella enterica, Yersinia pestis* and *Corynebacterium diphteriae*. The use of CRISPR-Cas as a direct antimicrobial tool has been studied recently. Artificial CRISPR arrays have been designed to kill pathogenic bacteria by targeting antibiotic resistance or virulence genes. This elegant way only aims for harmful strains in a bacterial population and allows nonpathogenic strains to overgrow the pathogens [20].

A recent study used lysogenic phages to introduce a CRISPR-Cas system in *E. coli*, which targets antibiotic resistance genes. The array was designed to additionally target the genomes of lytic phages leading to immunity towards phages only of antibiotic-sensitized bacteria. More precisely, bacteria that are unlikely to acquire antibiotic resistance genes due to their engineered spacer content are also resistant to lytic phages.

Thus, in the case of a phage infection, only pathogenic strains would be eradicated from the population. The medical potential of the CRISPR-Cas systems goes beyond antimicrobial treatment. The introduction of efficient and precise modifications into genes of an organism displays the basis for genome engineering. Programmable nucleases are used that specifically bind genomic regions and cleave the DNA at a desired position. Zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs) have been widely used to edit DNA. Both genome editing tools rely on the same principle: a sequence-specific DNA binding domain, which provides specificity, is fused to nuclease [20,21].

Owing to its simplicity, effectiveness and the possibility to target multiple genomic sites simultaneously, use of the CRISPR-Cas9 system is usually favoured over ZFN and TALEN systems. With the help of a targeting RNA the bacterial defence protein Cas can easily target DNA sequence. This single guide RNA (sgRNA) is an engineered hybrid of the naturally occurring tracrRNA: crRNA duplex and thus simplifies its application for genome editing purposes [21,22].

Repurposing the CRISPR-Cas9 system for genome editing exploits the DNA repair mechanisms of eukaryotic cells: after the introduction of a double-strand DNA break, the cell can repair the damage by non-homologous end joining (NHEJ). This process is error-prone and often leads to point mutations, deletions or causes frame shifts that alter the gene product and eventually abolish its function, which is favoured for genetic knockouts.

The CRISPR-Cas9 system has also opened the avenue for new animal models, including species that were previously dismissed due to difficulties in manipulation (e.g, the absence of meiosis and plasmids in *Candida albicans* is challenging for traditional editing methods).It has also made possible other studies that were previously unachievable [21,22].

Possible Limitations of the Cas9 system

Cas9 can be targeted to specific genomic locus via a 20-nt guide sequence on the sgRNA. The only requirement for the selection of Cas9 target sites is the presence of a point accepted mutation or PAM sequence directly 3′ of the 20-base pair targeted sequence. This PAM requirement does not severely limit the targeting range of SpCas9 - in the human genome. In addition to the targeting range. Another possible limitation is the potential for off-target mutagenesis [23].

Conclusion

CRISPR-Cas9 technology of genome editing helps in prompt and meticulous genomic manipulations which in turn screens numerous mutations *in vitro* or *in vivo*. The typical use of genetically reformed cells and animals has been an important tool in molecular biology since inception of the first knockout mouse model in the 1980s. Nowadays, the utilization of CRISPR-Cas9 technology enables researchers to mutate, silence, induce, or replace genetic component and simplify with precision, to explore gene functioning and the etiology of the disease.

Genome-wide association studies (GWAS) have recognized several numbers of loci corresponding to composite traits, mostly have low penetrance. The credibility to its precision and practicality is praiseworthy; the CRISPR-Cas9 technology can also be used to present the outcome of the phenotypic expression and polymorphisms [24].

Moreover, it may enable the researchers to modify numerous loci which is parallel to model composite phenotypes. There is a rapid increase in the interest of working on CRISPR –Cas technique over the years is noticed. The emphasize on various studies upon unexplored genetic and biochemical processes of the adaptive prokaryotic immune system requires more attention. the discrete CRISPR-Cas systems adaptability is remarkable and therefore with a current discovery of three newer types we can fully state the significance of CRISPR-Cas as a microbial defence system. However, highlighting on the other aspects of the antiviral system requires further cognizance [25].

The inclusion of newer spacers achieved by the CRISPR arrangement is still the most perplexing episode in CRISPR-Cas immunity. The level of conservation upon the biochemical basis of

a particular spacer addition among the various types have yet to be detected. For example, the role of supplementary proteins like Cas4 and Csn2 that have been shown which requires modification needs further exploration. The detection of the primed orientation has in type I CRISPR-Cas systems imparts a greater preventive edge towards mutated phages which had a tendency to escape CRISPR intrusion. The effect of CRISPR-Cas systems upon prokaryotic diversification is the most perplexing aspect. It has been noticed that the immune systems safeguard against phages and other MGEs that might possibly have valuable effects for an organism. In *E. coli* the CRISPR-Cas system is silenced by a protein called histone-like nucleoid structuring protein H-NS that gives a suggestion that sometimes a passive system is beneficial for a bacteria.

The interference of CRISPR-Cas systems with conjugation and transformation of plasmid without any intervention with competent bacteria may occur. Sometimes studies show negative occurrence of CRISPR-Cas systems association interfering with the amount of MGEs present in the chromosome, where evolutionary processes and horizontal gene transfer (HGT) is restricted. On the basis of evolutionary analysis contradictory results were found with no significant association between the number of HGT occurrence and CRISPR-Cas system. However, the evaluation upon the factors like predatory pressure, defence systems along with fitness costs and adaptive defence requires maintenance. Bacteria may immobilize CRISPR-Cas systems when there are plenty of predators. In such cases phage resistance due to, for example, mutations in receptor mutations seem more reasonable. Even though some oppositions remain but still CRISPR-Cas9-based genome editing methods will gain more appreciation with time and thus will be a useful technology in field of medicine.

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