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Review Article

CRIPSR Case System: Biological Role in Bacterial Virulence, Genome Editing and in Antimicrobial Resistance

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MR, MA, MK and AZD designed the research and collected data. AA, TA, NM and KK wrote the manusccript.

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Abstract | The aim of the present review was to study about the CRISPR Cas system and there role in genome editing, bacterial virulence and antibiotics resistance. CRISPR Cas system is an integral part of prokaryotic (Bacteria and Archaea) immune system that provides protection against viral infection. When bacteria recognize viral DNA inside it, bacteria incorporate small fragment of viral DNA into its genome at specific site termed as CRISPR locus. Insertion of viral DNA at CRISPR locus allows to remember, diagnose and clear the viral infection by the mechanism of sequence specific Adaptive Immunity. CRISPR Cas system is sustainable to combate with the mutation developed in viral genome that help viruses to escape from bacterial CRISPR Cas based immune system. CRISPR Cas system is a molecular mechanism of prokaryotic microorganism. It acts as bacterial natural adaptive immune system against phages, plasmids and foreign genomic elements. Mostly prokaryotes uses their CRIPR Cas system to enhance the integrity of their cell membrane that inhibit the permeability of antimicrobials from host body into the bacterial cells. CRISPR Cas system also help bacteria to evade from the host immune system by suppressing the activity of their immune receptors e.g. TLR. CRISPR Cas system also help bacteria to attach with the host body and replicate with in host body. It can develop antibiotic resistance in pathogenic bacteria, enhance their pathogenicity and can survival in host body.

Novelty Statement | CRISPR Cas system is an integral part of prokaryotic immune system that provides protection against viral infection and involved in genome editing, bacterial virulence and antibiotics resistance.

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Links Researchers

Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas system is an integral part of prokaryotic (Bacteria and Archaea) immune system that provides protection against viral infection. It is a molecular mechanism that consists of two major domains: DNA-binding domain that facilitates identification and attachment with sequence- specific DNA (Westra and Swarts, 2012). When bacteria recognize viral DNA inside it, bacteria incorporate small fragment of viral DNA into its genome at specific site termed as CRISPR locus. This short fragment of viral genome is transcribed into CRISPR RNA (crRNA) by bacteria. When crRNA combines with Trans activating CRISPR RNA (tracrRNA) a complex named as guide RNA (gRNA) is synthesized. gRNA guides Cas9 (a protein acting as endonuclease enzyme known as Cas9 to detect viral DNA for double strand breakage (DSB) (Figure 2c). When PM (Protospacer Adjacent Motif) sequences of viral DNA that matches with bacteria are recognized by gRNA, Cas9 nucleases cut viral DNA. Hence, viral attack is inhibited (Wang and Zhang, 2016). Use of Cas9 of streptococcus pyogenes the sequence 5'- NGG-3' is very common due to their wide acceptance by PMA. However, variation reported in Cas9 PMA sequences such as 5'-NGG-3' and 5'-YNT-3' affects the specificity of gRNA attachment with PAM (Esvelt and Mali, 2013; Kleinstiver and Prew, 2015).

Repairing of double strand breakages (DSBs) made either by the Cas9 enzyme or nucleases is done by Non-Homologous End Joining (NHEJ) method (Figure 2b). In this method small insertions or deletions (InDels) are presented at that site. Another method used to repair these Double Strand Breakages (DSBs) Homology directed repair (HDR) or Homologous Recombination (HR) method (Figure 2C) (Khan *et al.*, 2018). In HR method a donor DNA fragment that is homologous to the flanking sequences is used as template for repairing of DSBs (Cai and Fisher, 2016).

AIMS of this study

The present review was written to collect adapt from published authentic researches about the key role of CRISPR Cas system in bacterial immune system against viral infection (Horvath and Barrangou, 2010), development of antimicrobials resistance in prokaryotes (Horvath and Barrangou, 2010), bacterial sustainability with in host body by evading host immune system (Sampson and Napier, 2014), and pathogenicity of bacteria (Louwen and Staals, 2014).

History of CRISPR cas system

CRISPR Cas system was first described by Yoshizumi Ishino and his colleagues from of Osaka University in 1987. They found that bacteria encounter a phage DNA they insert a 32-nt (nucleotide) spacer sequence in into 29 nt repeated sequences in CRISPR loci (Cai and Fisher, 2016). After that, these repeated sequences were discovered in 90% of Archaea and 40% of sequenced bacterial genome. Function of these repeat sequences was still unknown (Horvath and Barrangou, 2010). Later on, many types of CRISPR associated genes adjacent to these repeats were discovered (Horvath and Barrangou, 2010). Since 2005, it is well known that actual origin of these special sequences is phage genome (Barrangou and Fremaux, 2007). However, it was hypothesized that this CRISPR Cas system can work as adaptive immune system for prokaryotes. Addition or deletion of spacer DNA that is homologous to the phage DNA can change the resistance of *Streptococcus thermophiles* to inhibit phage attack (Bolotin and Quinquis, 2005). Various facts of CRISPR Cas system were rapidly revealed by the discovery of crRNA, (Cas genes) CRISPR –associated genes, tracrRNA, and protospacer adjacent motif (PAM) (Mojica *et al.*, 2005).

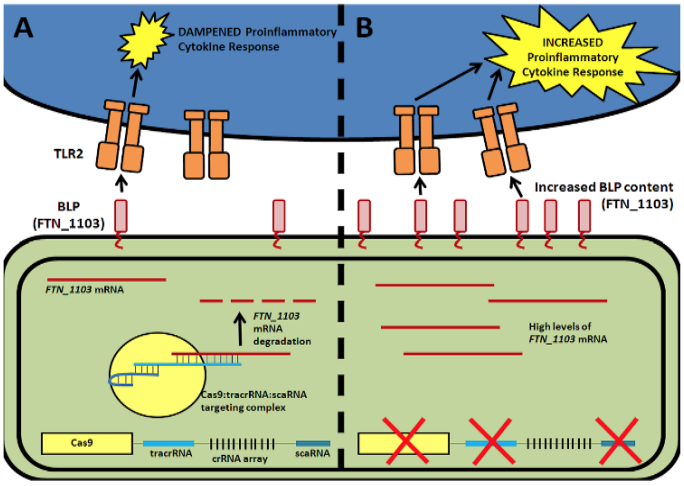

Figure 1: Cytokine response

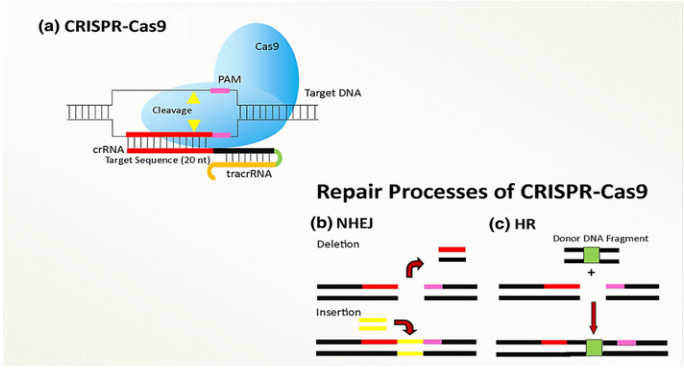

Figure 2: Mechanism by which foreign DNA is degraded by CRISPR Cas9 system.

Figure 1: Cytokine response

Composition of CRISPR cas system

CRISPR Cas system is composed of Clustered Regularly interspaced short palindromic repeats and linked Cas proteins. This system act as adaptive defense mechanism in prokaryotes (Briner and Donohoue, 2014).

CRISPR loci of this system consist on following three components:

Direct repeated sequences

Variation among the size (from 23 to 55 bps in length) of this region of CRISPR loci has been observed in different organisms. Clusters of these repeats are present on one or more loci of the same chromosome (Grissa and Vergunud, 2007). They are partially palindromic and have ability to construct hair pin. Usually, bacterial genome has three CRISPR arrays. While Archaeal genome contain five CRISPR arrays. A considerable feature of CRISPR array is the potential of their transcript to construct RNA secondary Structure (Kunin *et al.*, 2007).

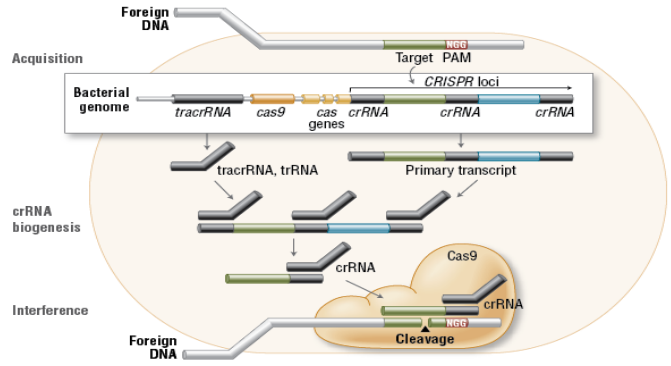

Figure 3: Genome editing applied to prevent atherosclerosis.

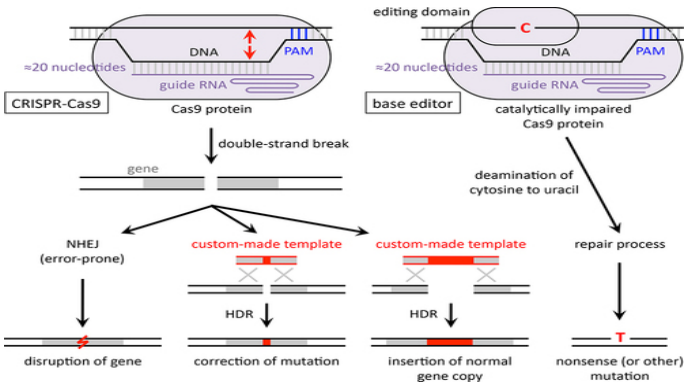

Figure 4: Genome editing.

Non-repetitive spacer sequences

Size of spacer sequences varies within a range of 26-72bps. Length of spacers present in a specific CRISPR array may be similar but identical spacer cannot be found within one specific CRIPSR array (Lillestøl and Redder, 2006). It is considered that these spacer sequences are of foreign mobile genetic elements origin, but sequence map of all known spacer sequences indicates that they contain very small portion of extrachromosomal DNA i.e. phage or plasmid DNA (Shah and Hansen, 2009). CRISPR analysis studies indicate polymorphism is number as well as kind of spacer sequences of various strains of specific specie. Hence, these spacer sequences can be used as a tool in epidemiological studies. CRISPRs can affect auto immunity because of spacers that contain ability to target self-genes. However, CRISPR can acquire autoimmune fitness that explain the plenty of degraded CRISPR system within prokaryotes (Stern and Keren, 2010).

Leader sequences

Leader sequences are AT rich and non-coding extend nucleotides region. These are situated at 5'-end between First CRISPR Loci and Last Cas gene (Jansen and Embden, 2002; Tang and Bachelleri, 2002). Leader sequences are similar in same prokaryotic species and different in different prokaryotic species (Jansen and Embden, 2002). It is said that transcription of CRISPR arrays begins in leader region because pre-crRNA of *Pyrococcus furiosus* is homologous to the 3'-end of the leader sequences (Hale and Kleppe, 2008). Modern researches provide evidences that leader sequences act as promotor for pre-crRNA. Leader sequences provide platform for attachment of Cas proteins needed for integrating spacer (Mojica and García-Martínez, 2005; He and Deem, 2010). Leader sequences have potential to construct an open transcriptional initiation complex and show their promoter activity in vitro as well as in vivo (Pul and Wum, 2010). Bioinformatics analysis of CRSIPR loci indicates that their ability to incorporate new spacer (He and Deem, 2010) and to execute the CRISPR expression and interference diminish if they don't have leader sequences (Marraffini and Sontheimer, 2008). The direct repeats and leader sequences are both conserved within same bacterial species but diverse in different bacterial species. Cas Genes CAR (CRISPER-Associated) genes are situated adjacent to CRISPR loci CRISPR containing Bacteria (Horvath and Barrangou, 2010). It is stated that CRISPR loci and CAS genes function is correlated i.e. in gene expression or DNA metabolism (Barrangou, 2013). According to Haft and His Colleagues (2005), there are 45 CAS gene families creating 6 core CAS families (cas 1 to cas 6). Out of these six core families' cas1 and cas2 are universal. They are the integral part of all CAS subtypes (Haft and Selenghut, 2005; Barrangou and Marraffini, 2014). Cas1 and 2 families contributes in the construction of repeat spacer insertion at leader end, new spacer acquisition and new repeat synthesis (Barrangou, 2013). Due to variation in molecular mechanism of action of CAS genes and their different phylogeny, three different types of CRISPR CAS system are present in prokaryots. Type one CRISPR system contain Cas3 gene. Type two CRISPR system contain Cas9 gene and Cas10 gene is present in type three CRISPR system (Makarova and Haft, 2011; Louwen and Staals, 2014). Some feature are common between type1 and type 3 CRISPR system but different from type 2 CRISPR system. Three novel subtypes of CRSIPR system has been discovered that contain addition cas genes along with conserved cas genes (e.g. cas1, cas2, and cas9). Cas2 is present as additional gene in type II-A subtype of CRISPR

Table 1: Bacterial species, type of CRISPR CAS system used and CAS protein.

Bacterial species	Type of CRISPR CAS system	CAS protein	References
Enterococcus species (*E. faecium, E. durans, E. hirae)*	Type II CRISPR Cas system	CRSIPR Cas 1 (Nuclease)	[42], [29]
Francisella, Parasutterella, Sutterella, Legionella	Type II-B system		[15]
Tistrella mobilis, Aizospirillum	Subtype III-A, I-C, II-C		[17]
Mycoplasmas, Planococcs, Antarcticus, Staphyloccus, Pseudintermedius, Staphylococcus lugdunensis	Type II-A	Cas9 genes	[14]
E. coli, Salmonella species, *M. xanthus, C.curves, C. ractus, C. concisus, Y. pestis, Pacnes C. fetus*	Type I CRIPR system	Cas3 (signature gene), Cas6(key protein)	[9], [18], [26], [44], [60], [45], [34],
N. meningitides, C. jejuni, S.thermophiles, L.monocytogenes, F.nivicida, S.pyogenes, *M. gallisepticum*, *S. mutants*	Type II CRISPR system	Cas9 (signature gene)	[22], [17], [35], [16], [34],
P. furiosus, S. epidermidis, M. tuberculosis	Type III CRSIPR system	Cas10 (signature gene)	[46], [1],

system. Type II-B has additional Cas4. While type II-C doesn't has any additional CAS gene (Makarova and Haft, 2011). Genes of Cas proteins are highly polymorphic. CAS proteins perform different functions at different stages of CRISPR mediated immunity. CAS proteins are classified into different families because of different numbers, arrangement and distribution (Marraffini and Sontheimer, 2010; Makarova and Haft, 2011). RRM (RNA Recognition Motif) is major functional domain of Cas protein. Sequence analysis of Cas protein indicates that it contain integrase, nuclease, polymerase and helicase domains. Presence of these domains in Cas protein predicts their involvement in DNA metabolism (Barrangou and Marraffini, 2014).

Classification of CRISPR Cas system

Classification of CRISPR Cas system is based on Cas signature genes and other related genes that mediate CRISPR activity. Each set of CRISPR system contain a different set of adaptation (spacer insertion), expression (pre-crRNA processing), interference (crRNA and target binding-cleavage) and Cas proteins i.e. Cas6 perform pre-crRNA processing; Cas8, Cas5, Cas7, and Csfi mediate crRNA attachment with target; Cas4, Cas1, and Cas2 mediate spacer insertion; target binding and cleavage is mediated by Cpf1, Cas 9, and Cas 10; Cas3 mediate target cleavage; while regulation of mechanism is controlled by Csn2. CRISPR Cas-system is divided into following types:

Type 1 CRISPR-Cas system

Cas3 signature protein or its mutants are encoded by the loci of type 1 CRISPR system. These signature proteins perform function of both nucleases as well as helicases. Type 1 system is divided into seven subtypes i.e. I-U and I-A to I-F. This system contains Cas1 to Cas8 proteins along with Cas3 protein. Various subtypes of Type 1 CRISPR system contain unique combination of Cas genes (Chaudhary, 2018).

Type 2 CRISPR system

This system is very simple because of its set of genes present in it. In this system crRNA binding with target and DNA cleavage is mediated by its signature protein called multidomain Cas9. 2-A, 2-B, and 2-C are the subtypes of Type 2 CRISPR system. All subtypes of type 2 CRISPR system also contain the set of Cas1, Cas2, and Cas4, and Csn2 genes (Dhawan and Sharma, 2015).

Type 3 CRISPR system

Cas 10 is the signature gene of this system. It also contains Cas7 and Cas5 genes. It has 2 subtypes: 3-A and 3-B. these subtypes may contain Cas6, Cas 2 and Cas1 genes (Dhawan and Sharma, 2015; Zhu and Klompe, 2018).

Other variants

Mostly bacteria contain type 4 CRISPR Cas system which contain a set of Cas7, Cas5 and Csf1 genes. Function of this system is still unknown (Zhu and Klompe, 2018).

The most advanced CRISPR Cas system is type 5 system that contain Cpf1 protein. This protein combine crRNA and form a complex that cut DNA and simplify the editing process (Luo and Lenay, 2016).

Biological role of CRISPR Cas system in bacterial virulence

It is suggested that CRISPR Cas system protects bacteria from foreign invaders and regulate bacterial pathogenicity by controlling their endogenous transcription. Bacteria (Francisella novicida) bypass host immune system because they contain Cas9 protein that inhibit the activation of TLR2 (Tall Like Receptor 2) (Shabbir ans Shabbir, 2019). TLR2 can recognize Bacterial lipoprotein, provoke pro-inflammatory response in host body and activate host immune cells to excrete bacteria out from host body (Mukherjee and Kamakar, 2016). It is suggested that type II CRISPR system have ability to suppress the BLP genes of bacteria (Barrangou, 2015).

Cas9, tracrRNA and small CRISPR associated RNA (sacRNA) regulate the suppression of BLP in F. novicida (Shabbir ans Shabbir, 2019).

Cytotoxic Chromosomal Targeting by CRISPR/Cas Systems Can Reshape Bacterial Genomes and Expel or Remodel Pathogenicity Islands

Many pathogenic and commensals bacteria possess Cas9 in abundance. Involvement of Cas9 in attachment of bacteria (Neisseria meningitides and C. jejuni) to host cell surface and replication with in host cells has been reported. It is suggested that virulence of *Legionella pneuomphila*, causative agent of Ligeonarries' disease, is due to Cas2 component of its CRISPR-Cas system.

Viral escape from CRISPR cas system

Bacteriophages have mutated their genome by deletion or insertion to escape from CRISPR Cas mediated immunity of Bacteria. According to Steel et al. (2013) acquisition and use of Bacterial CRISPR system to target antiviral defense mechanism by Bacteriophage is an evolutionary importance of this system (Qaisar *et al*., 2017). It is said that mutation in viral genome and sustainability of bacterial genome has been achieved by CRISPR Cas immune system.

Genome editing by CRISPR CAS technology

Group of technologies used by scientists to mutate the DNA of an organisms are termed as gene editing. These technologies permit genetic material to be inserted, deleted or changed at a specific location in genome (Hartung and Schiemann, 2014). CRISPR Cas9 is the best approach to be used for genome editing (Rizwan *et al*., 2016). It is naturally developed genome editing system of bacteria (Liu and Robinson, 2020). Bacteria recognize small fragment of phage genome and use them as a template to prepare CRISPR array (small fragment of DNA) (Nussenzweig and Marraffini, 2020).

These CRISPR array develop memory in bacteria against viral genome for protection in future invasion (Westra and Levin, 2020). Cas9 act as nuclease. CRSIPR Cas technology can be used for in vitro genome editing (Schulze and Lammers, 2020). Scientists synthesize a small fragment of RNA with short 'guide' sequence to that binds with a particular target sequence in genome. This guided RNA has ability to attach with Cas9 protein (Rizwan *et al*., 2016). These guided RNA helps Cas9 enzyme to recognize targeted DNA both *in vitro* as well as *in vivo*. Cas9 or Cpf1 enzyme act as nuclease and create Double breakages in targeted genome. After cutting DNA, Scientists use cellular DNA repair machinery to insert or remove fragments of genetic material (Huynh and Depner, 2020).

Genome editing capability of CRISPR Cas system has been adapted in bacteria e.g. *E. coli, Lactobacillus reuteri* (use in probiotics synthesis), *Streptococcus pneumonia, Clostridium begrinckii (use in alcohol production at industrial leve) and Streptomyces* species (use in production of antimicrobials) (Barrangou and van Pijkeren 2016).

The CRISPR-cas system promotes antimicrobial resistance in Campylobacter jejuni

Survival of bacterial pathogens with in host body depends upon integrity of bacterial envelope that counters act damage caused by membrane targeting Antibiotics (Hurdle and Onell, 2011). It is well known fact that CRISPR Cas system enhances the integrity of bacterial envelope and regulates its permeability. CRISPR Cas system of Prokaryotes provides them protection against invader viruses and foreign nucleic acids. It regulates the permeability of bacterial membrane is controlled by their CRISPR Cas system (Chen and Guan, 2016). It makes bacteria resistant to membrane targeting Antibiotics (Ahmad *et al*. 2019). Regulation of membrane permeability helps bacteria to resist recognition by multiple host receptors to enhance pathogenicity (Chen and Guan, 2016).

Conclusions and Recommendations

CRISPR Cas system is a molecular mechanism of prokaryotic microorganism. It acts as bacterial natural adaptive immune system against phages, plasmids and foreign genomic elements. It can develop antibiotic resistance in pathogenic bacteria, enhance their pathogenicity and can survival in host body. All these functions are performed by this system due to its genome editing capability. We can use this system (CRISPR CAS technology) to remove antibiotic resistance developed in bacteria. This technology can be used to treat several genetic disease i.e. cystic fibrosis, hemophilia, AIDS, etc. With the help of CRISPR Cas system we can edit genome of any organism.

Conflict of interest

The authors have declared no conflict of interest.

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