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Research Article

# CRISPR-Cas Systems in Bacteria: A Review of Mechanisms and Applications

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**Abstract:**

This review analyzes the development and various uses of the CRISPR-Cas systems in bacterial cells, emphasizing their function in adaptive immunity and their potential as effective tools in bioengineering. Consisting of arrays of CRISPR and P proteins, the CRISPR-Cas systems defend bacteria and archaea from viruses by targeting and destroying foreign genetic material sequences. Some of the literature on CRISPR systems subclasses has been reviewed, and basic stages of select bacteria practice modification over the nature and return of that genetic material are described. Class 1 and Class 2 types are particularly stressed, including Cas9 and Cpf1 proteins respectively which made a breakthrough in gene editing technologies. This paper also evaluates the recent trends in the CRISPR technology and its usage in genetic engineering, personalized medicine and diagnostics as well as the recent advances in gene editors, RNA targeting and strategically focused approaches towards antimicrobial resistance. Whether used in genetic stabilizing or cutting precise targeting, the review points out the off-target products and many other ethical issues associated with the techniques including human therapeutic applications in target genome editing. The overall aim of this article to provide guidelines to the researchers willing to employ the CRISPR-Cas systems and the genetic modification technology in developing better biotechnological products and therapeutic techniques in the future.

**keywords:** CRISPR-Cas systems, Bacterial immunity, Gene editing, Cas9 protein, Genome engineering



## 1. Introduction:

Here, we provide a brief description and necessary background concerning bacterial CRISPR-Cas systems, beginning with immunity and moving toward mechanisms. Focused genome engineering, regulation of gene expression as well as the use of CRISPR tools in exonuclease diagnostics received an esteemed award. Here we discussed exactly how these CRISPR based applications work and outline some key resources for those who are interested in obtaining an entry into this field. First, the four mechanisms of naturally existing Class 1 and Class 2 CRISPR-Cas systems are described in detail. The roles of the other large protein assemblies, namely Cas1-Cas2 integrase and multi-step cascades are utilized in the elucidation of the various forms of crRNA synthesis processes and the respective steps of Cas effector enzyme recruitment. Knowledge of these fundamentals is crucial since most engineered applications rely on the enzymes in a mode that is directly borrowed from natural immune systems.

In the next section, CRISPR-based genome editing applications, which utilize the DNA targeting

activities of various Cas enzymes, are described in detail, particularly focusing on how disease- or bio-manufacturing-relevant base editors utilize these enzymes. Next, we discuss RNA-binding Cas enzymes as tools for base editing, RNA knockdown, and diagnostics. Finally, we dedicate a section to strategies for re-engineering immunity and adding new functionalities to the naturally occurring systems by modifying the crRNA, Cascade, and Cas enzyme modules. This review should therefore serve as a comprehensive introduction to the sequence-specific aspects of almost the entire toolbox of CRISPR-Cas immunology and cutting-edge applications. The idea that CRISPR-Cas systems might be harnessed has transformed many areas of biotechnology and innovation and can guide fundamental research on viral growth and bacteria to understand viral and bacterial pathogenicity.

### 1.1. Background and Significance:

The human technologies scenario of the CRISPRs and Cas proteins have recently emerged in the molecular biology frontiers in the last years. In bacteria and archaea, these systems are one of the processes for natural immunity to bacteriophages (Nussenzweig & Marraffini, 2020). A pattern of reactions is set once a phage attacks. In other words, the cell system gives an "adapter," or an RNA sequence which is gotten from the parts of the virus genes. Subsequently, a Cas nucleases complex finds and cuts genes of this adapted RNA, mentioned earlier in the cell (Musharova et al., 2021). The cell prefers an infection defense by degrading the virus genome within it. Recent advances have successfully utilised the small system negatives as some of the important biotechnological assets. Research in this area has vast potential to transform the approach to research and medical therapeutics. The CRISPR system has recently continued to be of significant interest in various research on diverse platforms (Bhokisham et al., 2023).

This kind of CRISPR systems was first discovered in 1987 and the acronym CRISPR itself was coined in 2002. Hotta and colleagues proposed that horizontally transfer of CRISPR array was in 2005 and Kobayashi in 2007 described its ultimate key immunity role which provided the last piece of puzzle to infer that these elements are an interbacterial defense system through DNA (Mota et al., 2020). Cas proteins have been proposed to act as the judges exchanging short "spacers', of conserved virus and conjugated plasmid gene sequence since. The first cryo-electron microscopic results concerning the installation of the artificial spacers that has encoded the genes the paamivirus section in the CRISPR system had been reported in 2012 (Chen et al.,, 2020). However, after some months the result of that crucial research after some rounds, several laboratories started the generation of the CRISPR based successful research programmes for different applications. This is immediately evidenced by the fact that these systems are very efficient for in vitro cleavage of the HCV genome. From here, the CRISPR system focus began shifting not only from just becoming acquainted with bacterial immunities, but also utilizing these systems as exceptionally effective application in a broad spectrum of life science disciplines (Malhotra et al., 2021). But it is essential to note that pace of the development of CRISPR research is equally dependent on several front line research concerns (Davies, 2020).

## 2. Historical Development of CRISPR-Cas Systems:

Its identification in archaea and bacterias with relationship to Cas genes in mid 2000's have attracted much attention. Since then, numbers of species of bacteria and archaea for which CRISPR-Cas systems have been identified have been grown.Significant efforts have been made in the identification of underlying natures of CRISPR-Cas systems (Serajian et al., 2021). has generated much excitement. Several species of bacteria and archaea that contain CRISPR-Cas systems have been isolated since then. There has been intensive research to unravel the basic properties of CRISPR-Cas systems (Serajian et al., 2021). Some of the model CRISPR-bearing species are human pathogen and industrial strains belongs to Escherichia, Mycobacterium, Staphylococcus, Streptococcus and Listeria.Cas proteins, including Cas9, have been employed as effector to modify bacterial, archaeal, and eukaryotic genomes, and the proteins had been utilised in laboratory experimentation to neutralise virus infections (Rizwan et al., 2021).ISPR-Cas systems have been isolated since then. There has been intensive research to unravel the basic properties of CRISPR-Cas systems (Serajian et al., 2021). Some of the model CRISPR-bearing species are representatives of human pathogens and industrial strains from the genera Escherichia, Mycobacterium, Staphylococcus, Streptococcus, and Listeria. Cas proteins, including Cas9, have been used as tools to edit bacterial, archaeal, and eukaryotic genomes and have been used in

laboratory studies to inactivate virus infections (Rizwan et al., 2021).

It is not clear when CRISPR systems in archaea and bacteria were recruited for maintaining immunity to mobile genetic elements. One group of researchers proposed that diversity-generating retroelements were the precursors of CRISPR-Cas systems (González-Delgado, 2021). DGR introduce polymorphisms in target proteins to help viruses escape from attack by selecting resistant host cell clones. It is proposed that some specialized transposon or a retroelement that could not carry a gene cassette, probably because of its arrangements or location relative to another mobile genetic element in the host genome, clustered in an array adjacent to the affected gene. Those elements that were the most successful were those that expressed RNA and protein that helped to redirect those elements, or others yet to enter the array, away from unique genes and those that helped to inactivate the elements themselves. Subsequently, locus expansion through either duplication of neighboring spacers or additional transposition events contributed to array expansion (Egido *et al.*, 2022). However, all the genetic elements related to DGRs that have been identified so far are forms of retroelements, which have been known to be present only in eubacteria and not in archaea or bacteria (González-Delgado, 2021).

### 2.1. Discovery of CRISPR Arrays:

The name CRISPR stands for clustered regularly interspaced short palindromic repeats which refers to the genetic organization that this sequence occupies. As with the genes they are intercalated with, CRISPRs have been found in approximately 40% of bacterial and more than 80% of archaeal genomes to have been sequenced. Nevertheless, the specific roles of this genetic system remain unravelled only recently (Münch et al., 2021). Previously, CRISPR arrays of Streptococcus spp harbored only limited repertoire of Pgl and were of shorter length than typical when present (Smith, 2020). After this, contributions to the antimicrobial network against viral pathogens were published (Schultz et al., 2022). Identification of what is now known a CRISPR array in Halobacterium, the sequence pattern of which was interrupted at the locus at regularly spaced intervals. Originally, spacer sequences were linked with viruses suggesting the array involved in adaptive immunity associations (Alonso-Lerma et al., 2023).

These data were identified by other authors to begin making basic discoveries about the CRISPR arrays. As an adaptation of the arrays in five distinct bacteria, the array spacers were analyzed and compared to phage and plasmid DNA (Koonin & Makarova, 2022). This suggests that the CRISPR array and the proteins recognized together with the array are involved in antiviral defense in the bacteria that host the array (Mortensen et al., 2021). In addition, these studies pointed to an involvement of the CRISPR-associated proteins in the function of the CRISPR arrays. Studied an array of Thermus thermophilus HB8 that is one of the most numerous spacers observed in the natural bacteria (Shmakov et al., 2023). Provided reports of the spacers of 29 archaeal and bacterial organisms, evolution and conservation of the system. These studies investigated the spacer sequences bioinformatically and the results indicated a association of spacer presence and flanking arrays by Payne et al 2021.

## 3. Mechanisms of CRISPR-Cas Systems:

The CRISPR systems of Bacteria and Archaea are called adaptive immune systems to defend themselves from viral infections. The adaptive immune response of these prokaryotes is divided into three main phases: Protection, creativity, and regulation (Garcia-Robledo et al., 2020). The CRISPR loci in the host genome contain its guide sequences which is the part of the invasive DNA from the infecting viruses; the guide sequences enable an endonucleolytic destruction of specific viral sequences complementary to the guide sequences on the viral genome inhibiting expression or replication of the viruses. The integration of invader DNA sequences into the host CRISPR arrays occurs in the attachment phase and, in the subsequent phases, corresponding RNA transcripts are synthesised from integrated sequences by certain promoters present before the repeats (Lin et al., 2021). The CRISPR transcript is cut into small crRNA guides, and one of the several types of CRISPR effector complexes targets the infecting DNA, depending on the crRNA base sequence, to enable adaptive immunity and selection against the present viral infection, although it may not protect against reinfection by the same virus in the future. Muta gene sis and evolution tat have always been present have enhanced the bacterial adaptive immunity (Nus sen zew ig & Mar raffini, 2020).

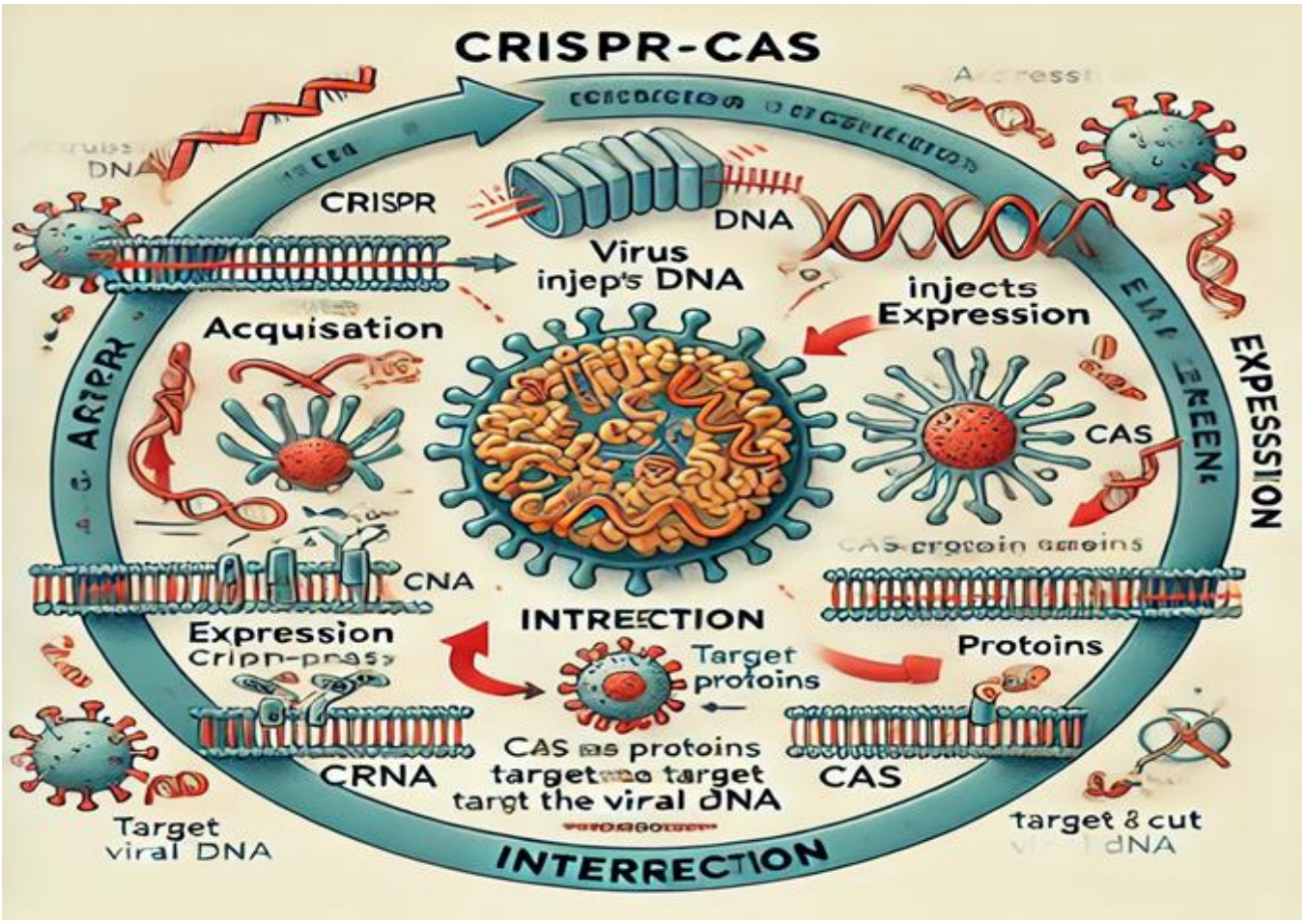


**Figure 1: The mechanism of CRISPR-Cas Systems: The above given is a schematic diagram which shows the account of the CRISPR-Cas that helps the bacterial cell to function as an immune system. It is carried out in three steps, namely: Accomplishment which involves incorporation of viral DNA into bacterial DNA; Second, transcription whereby the bacterial CRISPR array is transcribed into crRNA; Lastly, inhibition, where the crRNA brings the message of the viral DNA to the Cas's which then degrade it to avoid any infections. In this way, the bacteria manage to achieve the ability to recognize such viruses and defend against the next ones**.

One of the major factors that define the ability of CRISPR-Cas against viruses is the be the binding and association between crRNA and effector Cascade complexes that work to deliver crRNA to its target sequence in the viral DNA. Cascade is usually composed of at least one trans-activating CRISPR RNA molecule and a Cas protein complex (Javaid and Choi, 2021). The crRNA:tracrRNA duplex directs Cas proteins to the DNA sequences in the infected host genome, and the subsequent interaction between crRNA and Cas proteins define the interference profile (Liao & Beisel, 2021). Actually, the CRISPR machinery is well understood as well as the viral immune system. The general mechanisms and analyses of biological information are required to explain the capacity of CRISPR to learn the novel viral sequences and enhance the disease techniques like targeted genome modification (Bayoumi & Munir, 2021). The natural variation of spacers, the sequences used by bacteria to identify target regions in viral and plasmid DNA, can be used to assess bacterial genetic variation. What's more, spacers help defend bacteria and Archaea from this and other infections through innate acquired immunity memory and are a target as they record invasion by parasitic DNA (Zhang et al., 2022).

### 3.1. CRISPR Array Structure and Function:

The feature that set apart different CRISPR-Cas systems is the repeat-sequence of the so-called CRISPR array. This genetic component is direct repeats separated by distinct sequences that are called spacers because of their origin from mobile genetic elements such as viruses and plasmids (Pourcel et al., 2020). To protect against diverse types of pathogen attacks, the bacteria also have the capability to collect new spacers from these attackers and since their transfer, bequeath their obtained flexibility to successive generations of bacteria (Pfeifer et al., 2022). Inheritance and variability of the spacer play the key role in

forming the bacterial evolutionary identity. More recent members of the spacer family will be inserted into the proximal portion of the array and are placed the DNA sequence, the leader of the array (Zhang et al., 2022).

The repeat part of the sequence of spacers from the leader ends is transcribed into tandem CRISPR-RNA which in turn is disaggregated into RNA sub-sequences each of which possess a spacer. As part of the immune mechanism, CRISPR arrays are transcribed from spacers into a simple piece of RNA containing only these repetitions, each a repeat and a section of space separated by short repeats (Joberty et al., 2020). The formation of spacer1 pre-crRNA takes place undergoes in principle, in up to two steps, by either transcription of entire arrays of the basic units in one primary transcript and subsequent processing of segments of the complex via multiple copies of the repeats or the appearance of non-capped CRISPR RNA molecules that can be synthesized at the end of the primary transcript. Thus, the RNA segments, containing copy of the unique spacer together with bordered repeat can be referred to as mature cr RNA.

## 4. Types of CRISPR-Cas Systems:

Four types of CRISPR-Cas systems have been identified on the basis of their signatures: characterised by (i) system class according to attributes such as multi-subunit proteins or mechanics, (ii) the protein composition of the system, (iii) the process of adaptation and/or interference as the mechanisms used, and (iv) distribution concerning other systems or their evolutive relatedness (Makarova et al., 2020). According to the system class and overall characteristics (e.g., protein arrangement, interactions, depending on the types of complexes involved, and strategies inherent to the process), mechanisms and function of the system, the CRISPR-Cas systems have been classified into two major classes: Class 1 and Class 2. The Class 1 system is divided into six types of systems depending on diverse molecular machinery: sequence- or structure-specific endonucleases and other proteins related to interference complexes for RNA-directed DNA interference. For its multiplex interference, Type IV is categorised according to how the Class 1d system modulates its effector protein.

Class 2 systems, especially those more investigated for their gene editing capabilities, are typical of simple single-protein systems where a single RNA molecule directs Cas to DNA sequences, such as Cas9 and Cpf1 ( Özcan et al.,, 2021). Due to the versatility of the type II CRISPR-Cas9 system across a wide spectrum of function such as gene editing, regulation, imaging, modulation of gene expression, and of perturbation screens both in biotechnology, synthetic biology, basic and bioprospecting research, these simpler system proteins have been classified into the same class based on mechanistic and technical similarities (Mota et al., 2020). Type III and type VI systems are located in Class I; type III is more related to Class I and the effector of Type III has some features of Class 2 but regulates the processes of Class 2. Type III is incorporated in Class 1 owing partly to its structural aspects and nature of a Class 1 system. Although that has important repercussions in applications and practical exploitation, especially for genome editing, there may be discovery or mechanistic applications of candidates on what we know is an operation of their cellular systems (Makarova et al., 2020).

To better understand this classification complexity, a description of the distinguishing features of each CRISPR-Cas subtype is offered here. In Class 1, many different cas genes are linked to other forms of cas genes that belong to the same family, where the corresponding genes encode multi-subunit complexes (Taylor et al., 2021). The current findings show that there are differences of the nuclease domains in the effector proteins of the systems under study. This diversity involves targeting of such elements as endoribonuclease and several accessory domains which regulate nuclease function of the latter. Helicase domains and cyclobutane pyrimidine dimers, intermembrane protein for instance, are also found in some genes (Munawar & Ahmad, 2021). Personal properties of each system associated with Biochemical and mechanistic working framework define the application of Type I, Type III, and Type IV such as in biotechnology, synthetic biology or in gene-editing technologies where Cas proteins are used in enzymes Liu et al., , 2020). Albeit, there could still be high cost of identifying or manufacturing a new effector for utilization in biotechnological applications, the existing and increasing searches and studies mean to discover the mechanisms and to harness those new toolkits with new function (Zhu et al.,, 2020). This way, such studies help to establish that gene editing will become more specific, invasive, and precise eventually opening the way for safer gene-editing systems.

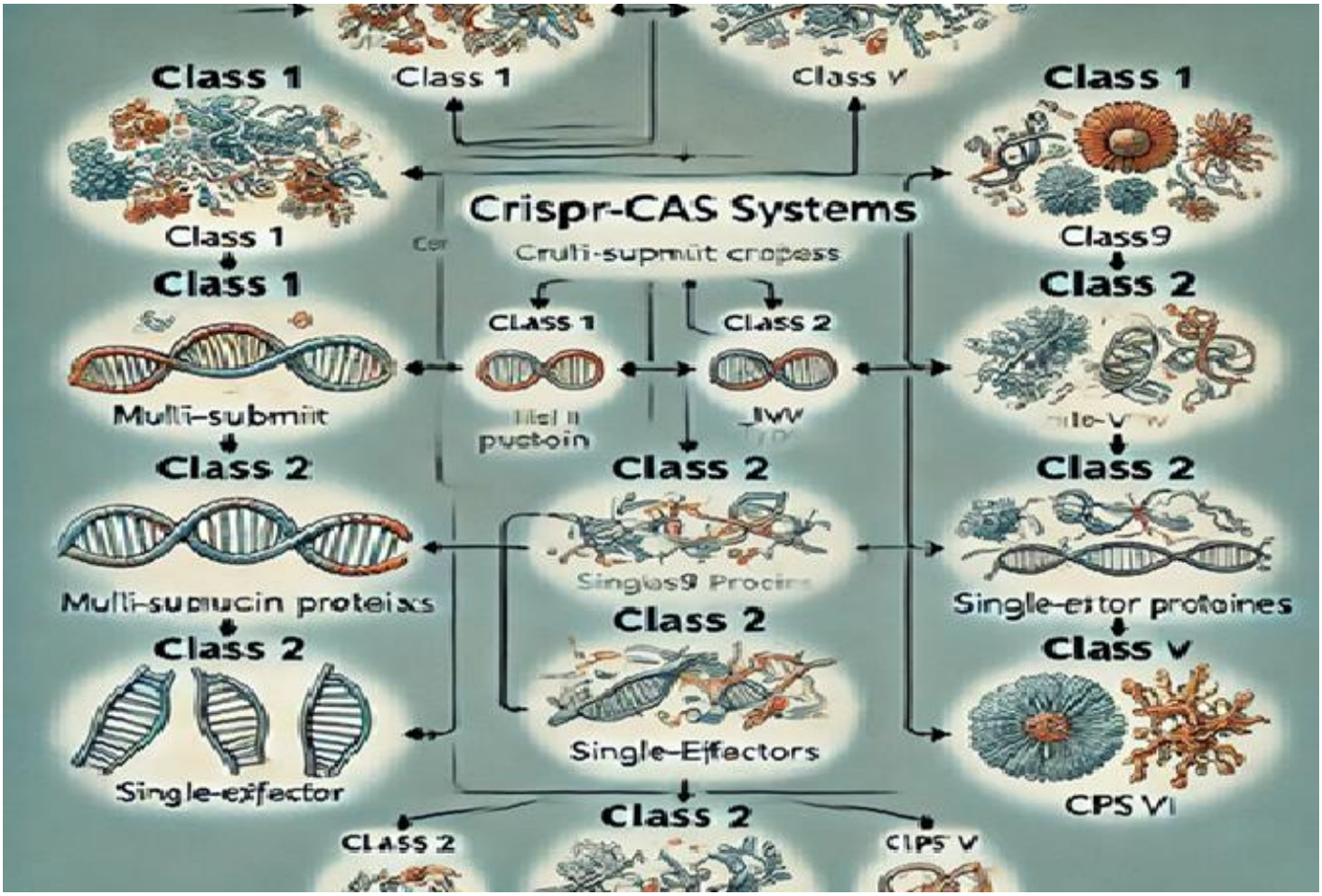


**Figure 2: The presented flowchart shows that there are two main classes of CRISPR-Cas systems that function more than one protein complex, which are type I, III and IV encoding complexes and other single effector proteins including Cas9 and Cpf for type II, V and VI respectively. The structural distinction between these two classes is, that Class 1 comprises numerous proteins that may interact with DNA but Class 2 consists of but a single protein which can interact with DNA.**

### 4.1. Class 1 and Class 2 Systems:

Sforza et al originally distinguished two types of CRISPR-Cas systems based on similarities in the genes encoding these proteins and some differences between them, and indeed these systems are quite different in structure and function. Class 1 systems, which are subdivided into 4 general types, have an overlapping Cas effector complex of Cas proteins, while Class 2 systems possess only a one-protein crRNA biogenesis and crRNA-directed interference module (Makarova et al., 2020). Unlike Class 1 systems which frequently display multiple Cas protein ratios in the effector complex, Class 2 systems have a single effector protein (Chaudhuri et al., , 2022). This class organization also make Class 2 a particularly important tool in biotechnological research, especially for type II CRISPR system. Class 1 systems thus involves higher risks to other usage than the other more specific Class 2 systems (Mota et al., 2020).

Class 1 system is described by several effector proteins containing a huge crRNA-Cas effector architectural structure as an effector core that can alter definite conformation for matching complementary pair connection with the invasion DNA target and DNA scission or prevention (Bhatia, Yadav, 2023). The Cas1 and Cas2 integrase genes are used in most prokaryotes with adaptive immunity by CRISPR-Cas for the acquisition of CRISPR spacer. It is general to observe that Class 1 systems may be more complicated than Class 2 systems due to the variety of Cas proteins that are presented in former systems: some evolutionary work on systematizing and cataloging that complexity encompasses more crRNA repeat priming pathway proteins than in the latter (Budhathoki et al., 2020). Class 2 systems, to this date considerably less complex than Class 1 systems, incorporate a single crRNA effector protein complex with Cas9, Cas12 or Cas14 found in organisms ranging in size and shape. Restricted re-targeting is evident in Class 2, which is different from Class 1; it is evidence that, Cas9 and Cas12

can re-target effectively with such crRNAs (Özcan et al., , 2021).

## 5. Applications of CRISPR-Cas Systems:

### 5.1 Applications in Genetic Engineering and Biotechnology:

This approach is restricted by the primary development of multiple mycobacterial genera and tick species. It has enabled the application of focused enzymes in a plethora of living organisms such as; plants, animals, microbiological cells, and ticks. Thanks to the use of this tool, exercises like marking sequences, protein change, cell alteration, editing animals, and base modifying, and RNA transposition are possible (Deckers et al.,, 2020). In addition, you may use any significant or new introduced catalyst to evaluate the target component regarding the said deletion transfer from DNA segment to a sequence component. Such study might suggest the potential of editing across a wider range of scripts of the body (Li et al., 2020).

### 5.2 Precision Gene Editing of Microbes:

In the past decades, a plethora of genetically tractable organisms has been engineered, and many of the working concepts of the CRISPR system have been executed biophysically in bacteria. Although extensive experimentation has revealed that just a few CRISPR-Cas systems are ineffective in archaea and eukaryotes and have therapeutically edited a range of animal cells (Liu et al.,, 2020). It has, as has already been thoroughly reviewed. CRISPR based technology has been used to editing and rewiring the plant and subsidiary bacteria only in recent years (Khan et al., 2022). Some key features of the molecular organisation of the CRISPR system were outlined together with the linguistic coherence and diversity of the CRISPR systems (Wang et al., , 2020).

### 5.1. Gene Editing and Genome Engineering:

Gene editing and genome engineering are the general terms used to describe a group of methods that may be applied to modify genetic information at various tiers (Boti et al., 2023). The CRISPR-Cas9 system-based gene editing and genome engineering has grown rapidly popular in genetics and genomics since its appearance. This is because the CRISPR systems can fulfill very effective and selective genetic works such as knockout, insertion, point mutation, and Genetic Defect Correction of mutated or missing genes (Nadakuduti & enciso-Rodriguez, 2021). CRISPR systems are up to 50% cheaper than molecular scissors ZFNs and TALENs, and all CRISPR systems are equally efficient. Moreover, several studies have shown that the CRISPR system is less complex as compared to ZFNs and TALENs in design, handling for gene editing; thus, for the first-time users, CRISPR system is the tool of choice (Ali et al., 2023). The flexibility of the CRISPR-Cas system is not merely limited to the genetic modification; more so, it serves a broad use in examining gene functions and variations in different organisms (Chaudhuri et al., , 2022).

CRISPR-Cas9 has been used in gene editing and targeting multiple sites simultaneously. Some of the CRISPR-Cas9 gene editing case studies include studies on loci of desired genes in animals, plants, and microorganisms in response to their behaviors when manipulated (Wang *et al.*, 2022). It should be noted that, until now, the clinical applications of CRISPR gene editing in human therapeutics and germline modification have raised many concerns (Schleidgen *et al.*, 2020). Gene editing mediated by the CRISPR system is highly programmable in a sequence-specific manner, but the off-target impacts are slightly less predictable and controllable. Off-target issues need to be further investigated to achieve a low error rate, especially in the treatment of some complex genetic disorders (Naeem *et al.*, , 2020). Additionally, the molecular delivery and issues of base editing in genetic manipulations still need to be optimized. It is clear that ethical and authenticity issues should be cautiously considered before DNA alterations are authorized for clinical and therapeutic purposes (Alpaslan-Roodenberg *et al.*, 2021). Ethically, the use of CRISPR gene-editing systems in the human germline is not only a moral issue but also a significant social issue (Piergentili *et al.*, 2021). Furthermore, although the CRISPR tool has changed the way geneticists work in the field of genetics and serves as a general tool for researchers, like ZFNs and TALENs, other genetic engineering tools, such as some approaches mediated by the CRISPR tool that alter gene function without changing the stored DNA sequence's C and G or base, are not within the scope of this topic (Li *et al.*, 2023). Future studies need to further optimize genetic manipulation and have not been reviewed here.

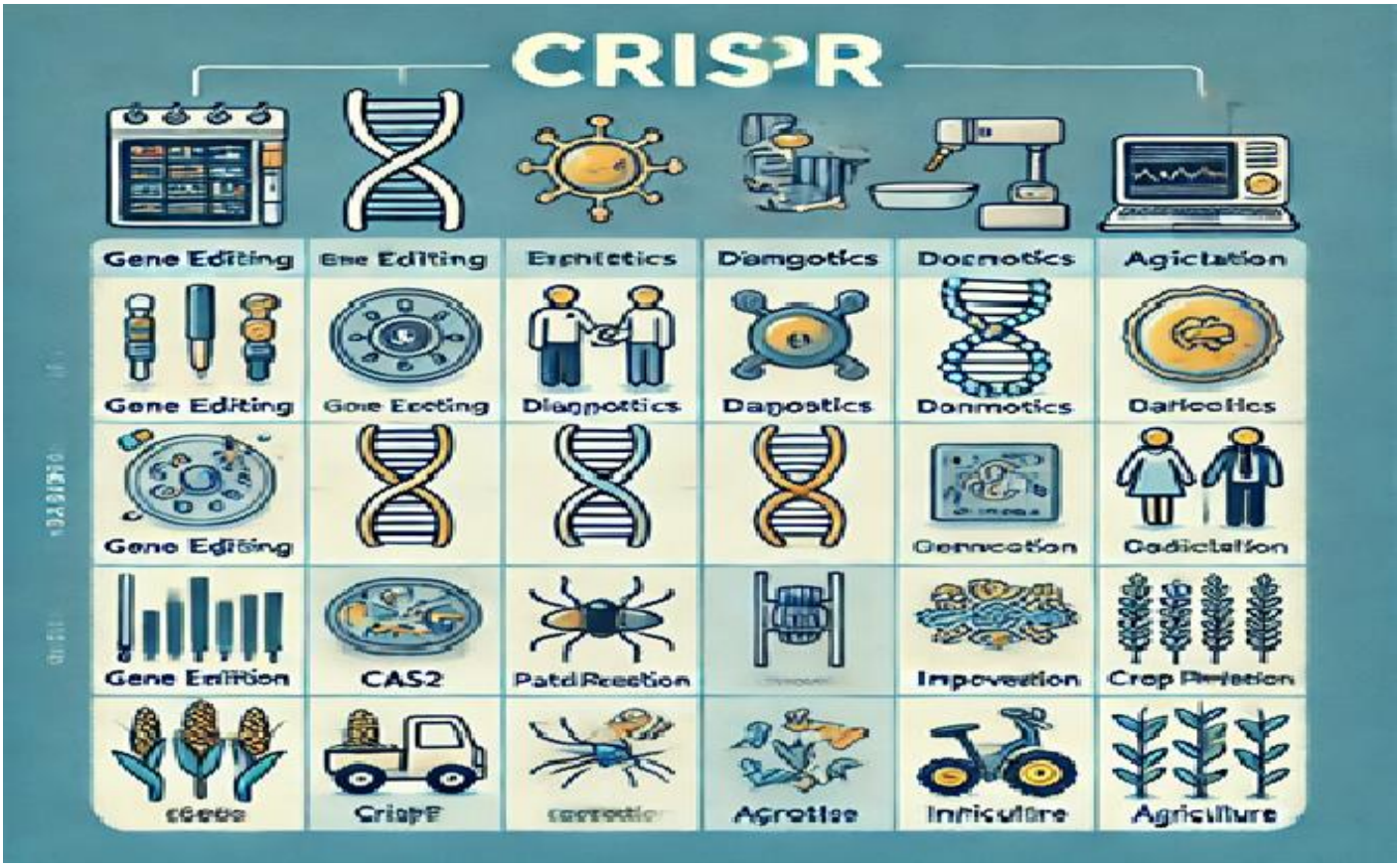


**Figure 3: The comparison of CRISPR-Cas processes in medicine and biotechnologies' domain. This is a figure that is meant to quickly compare virtually all of the CRISPR-Cas technologies by their sectors such as, gene editing, diagnostics, gene regulation, agriculture etc. It also tries to point out which particular CRISPR-Cas geometry was involved in those operations such as Cas9 and Cas12a in disease treatment, and crop improvement operations to illustrate the adaptive nature of CRISPR towards the development of medicine and biotechnological approaches.**

## 6. CRISPR-Cas Systems in Bacterial Immunity:

While many bacteria can defend against viruses and plasmids using restriction-modification systems and the ability to eliminate foreign DNA, most bacterial species contain adaptive immune CRISPR-Cas systems that are akin to the adaptive immunity response of eukaryotes (Newsom *et al.*, 2021). Like immune systems in metazoans, CRISPR-Cas systems also have adaptive immunity. Bacteria use the CRISPR-Cas systems as a form of protection against mainly phages, given that viruses are encountered more frequently by bacteria than plasmids (Fillol-Salom *et al.*, 2020). They adapt the systems for added protection. The first of the two main processes of CRISPR-Cas immunity is the acquisition of new spacers from the invading genetic element. This functions to broaden the host's immune repertoire. The second process, called 'interference', functions to target and destroy the foreign DNA (Nussenzweig & Marraffini, 2020).

CRISPR-Cas systems are categorized into various subtypes depending on the organization of the system and components thought to play major roles in immunity. Types I, III, and IV contain multiple subtypes in themselves, and type VI contains two. Each subtype contains signature proteins that can be identified and used to determine subtyp (Makarova *et al.*, 2020)e. They all act in the two interference processes. The events of the environment have led to the evolution of the bacterial genome to create protective barriers for themselves. Regulating time and resource investment allows for a more flexible barrier. Bacteria faced many threats, but en masse, viruses infected at the highest rate and an escalating rate. CRISPR systems allow for bacteria to adapt to the threats that are the most prevalent and reproducing most rapidly. An additional result of CRISPR arrays being used to protect against phages is the creation of a historical record (Malone *et al.*, , 2021).

### 6.1. Adaptive Immune Response:

Adaptive immune response is the hallmark of CRISPR-Cas systems. CRISPRs are arrays of short unique sequences of spacers interspersed between

conserved short repeats. Spacers are normally viral or plasmid in origin. A consequence of bacteria acquiring spacers from an infecting virus during infection is that the bacterium now has an immunological memory of that virus in the form of a spacer (Ganger *et al.,* ). The presence of this spacer in the genome of the bacterium gives it a selective benefit leading to evolution towards resistance to the viral infection that must naturally, in order to be successful, re-infect that cell. The bacterium has a unique selective advantage from the other bacteria due to having this new spacer because the bacteria without the spacer will be killed by phages, while the mutant with that spacer will not (Cornuault *et al.,* 2020). This means that the bacteria that receive spacers first undergo a significant adaptive radiation and come to dominate the population. During the adaptive phase, an entirely new set of Cas genes that are foreign to the organism evolve. Adaptive Cas genes are driven by variation in spacer sequences that are necessary for phage protection (Bonsma-Fisher, 2022). Upon infection, the immune state of the bacterium is checked. This check involves expression of the repeat spacer array (now in the form of pre-crRNA), processing of this pre-crRNA transcript into smaller units (crRNA) that contain only one repeat and one spacer, and combining these crRNAs with Cas proteins into functional crRNA-Cas complexes. In the event of a mismatch between a crRNA and a segment of phage DNA, the crRNA-Cas complex cuts the DNA to neutralize the phage (Fage *et al.,* , 2021). Once the phage is neutralized, the crRNA is returned to the host's genome, along with any subsequent acquisition of new DNA segments. Upon cell division, one daughter cell will have the crRNA while the other daughter cell will not. When those daughter cells give rise to a population of cells, the ones that don't have the crRNA will eventually die when infected by new phages. The one that does have the crRNA will survive the infection and go some way towards replenishing the healthy population of bacteria (Gao *et al.,* 2020). Different bacteria handle the immunological memory in different ways. For instance, some cells only maintain the immunological memory for a few days while other bacteria can hold an immunological memory for over 20 years (Sharrock & Sun, 2020).

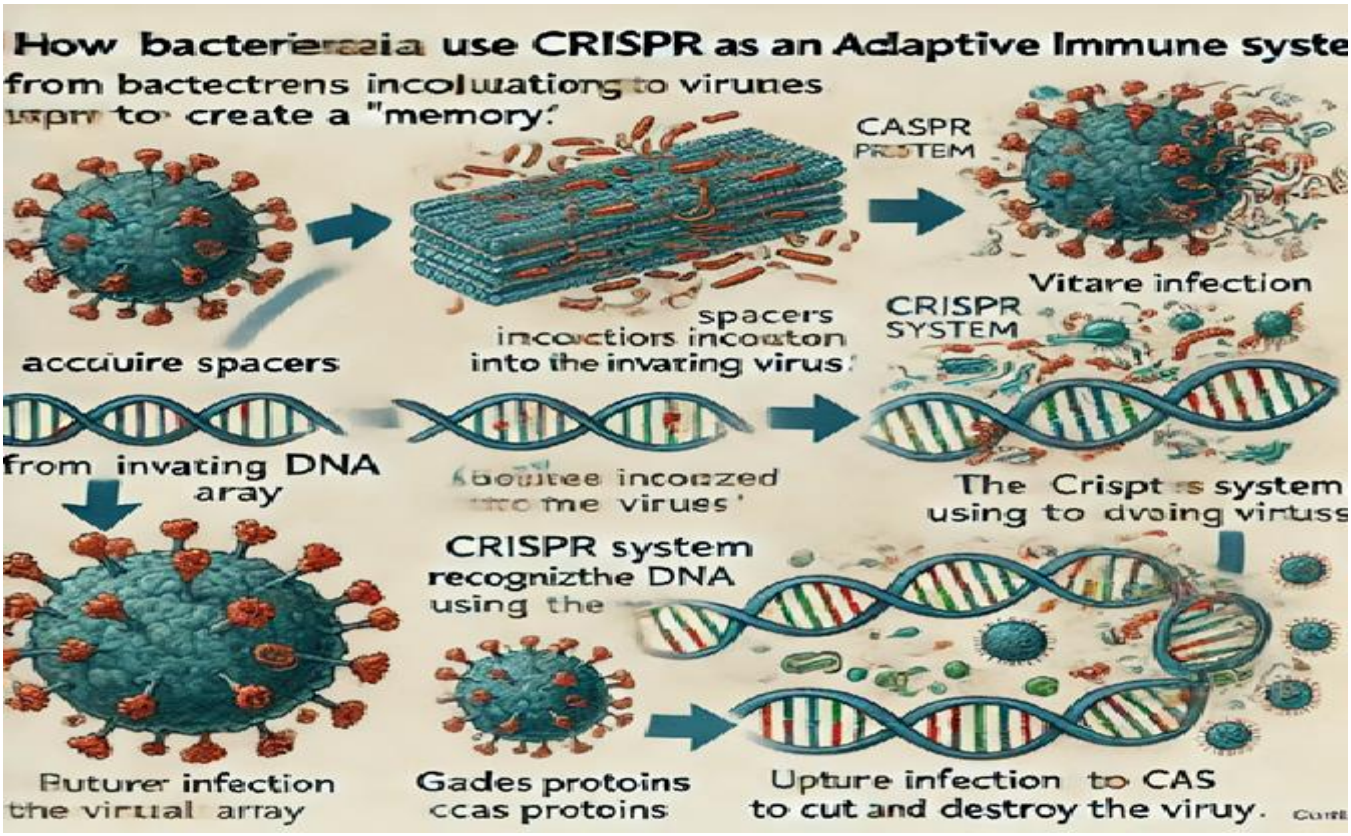


**Figure 4: CRISPR Adaptive Immune System. The schematics of the CRISPR-Cas system present within bacteria as an adaptive immune response. During the first infection, the bacterial cell captures the invader by incorporating part of the invader's DNA (spacer) into its CRISPR array, which becomes somatic memory. Then when the same virus invades in the future, the bacterium uses this memory to direct C.**

### 7.1 Current Challenges and Future Directions:

CRISPR research is currently blooming; however, a lot of technical and biological questions remain unanswered. Technical issues regarding CRISPR research mainly revolve around on- and off-targeting, delivery methods, and the enzymes being used. Off-target effects can currently be accounted for in CRISPR-fueled equipment by using additional CPUs to screen for these mistakes, while novel enzymes are being investigated (Naeem *et al.*, , 2020). However, it is still not well understood how to properly deliver the system to each tissue because of different host defenses, nor is it clear that the most efficient, precise, environmentally friendly host-defense mechanism has been discovered (Vedadghavami *et al.*, , 2020). Ethical challenges include but are not limited to the societal impact of the recent development of editing the human genome in a single-celled embryo and releasing it into society, as well as the widely recognized possible implications of modified organisms on the environment (Zhang *et al.*, 2020).

There are numerous clinical and emerging applications in CRISPR if these challenges can be overcome. Its applications currently include medicine, specifically the treatment of genetic disorders (Serajian *et al.*, 2021). CRISPR is also currently being researched in agriculture with the goal of creating a better supply of crops and developing more productive, valuable greenhouse plants (Rajput *et al.*, 2021). In the near future, experts across various fields are planning to work with CRISPR in research to solve global concerns, such as drug and packaging issues (Serajian *et al.*, 2021). As with any novel technology, rigorous exploration should be conducted before any clinical research is performed to define both the short-term and long-term consequences. Also, proportional regulatory frameworks should be developed worldwide to guarantee that CRISPR is used in a secure manner (Uddin *et al.*, , 2020). In sum, austere ways throughout various areas are absent. These challenges, once addressed, should assist the CRISPR community in recognizing the full potential of these microbes in the foreseeable future (Arroyo-Olarte *et al.*, , 2021).

### 7.2. Ethical Considerations:

CRISPR systems have created massive interest and attention worldwide over the past decade. This has resulted in breakthroughs in the development of gene editing technologies, microbiome manipulation, and gene drive systems, among others. This section addresses ethical considerations related to CRISPR technology, and, in particular, applications in gene editing and biotechnology.

Altering genetic characteristics comes with a range of moral implications across all applications, and manipulation in humans can carry significant and far-reaching consequences for individuals, their offspring, and communities. This is especially true given that germline gene editing technology could lead to changes that will be passed on to future generations (Lewens, 2020). Ethical concerns surrounding the use of CRISPR technologies in biotechnology range from the misalignment of the expressed and desired traits of a genetically engineered organism to potential effects on human and animal health, as well as the health of ecosystems. The use of biotechnology may also incite concerns regarding the possibility of technology misuse, such as developing designer babies or creating bioweapons (Piergentili *et al.*, 2021).

The advanced use of CRISPR gene editing technologies in biology may be more personal and public benefit that taking place in circumstances with the intention of addressing inherited genetic disorders; addressing the prevalence of infectious diseases; healing cancer; defending against environmental toxins, and various diseases (Kan & Doudna, 2022). But it also still poses a threat to increase existing disparities in heath care and questions the reach of the patient's consent by utilizing certain technology such as CRISPR-Cas (D'Souza et al., 2024). In addition, gene editing is taking place in socio-economic contexts that enable and define scientific possibilities and realities of science as well as technologies of science. Inequality is expected to affect the availability of the interventions and the technologies based on CRISPR since resource distribution is usually skewed. Responsible conduct of research as well as the institutional rules, norms and mechanisms can act as the form of governance to address ethical risks (Subica, 2023). Further, requirements, which are constructed as oversight and governance mechanisms, can encompasses such aspects as parent consent or state approval in the interest of the most vulnerable participants, including children and adolescents. Explaining these opinions will add to burdensome scientific disputes regarding cloning of humans and germline interference. They also complement ethics debates on scientific research involving human embryos and stem cells and genetic instruments and methods.

Given the perceived power embedded in recent scientific discoveries and applications that make use of CRISPR technologies, stakeholders must engage in more transparent public dialogue. This practice will accompany the development of regulations that fit with societal values and beliefs. Tied to responsible research practices and oversight is the labeling of controversial topics, which can invite and encourage cross-disciplinary research and collaborations. Dual-use research of concern can also inform public dialogue and governmental oversight. This ethics paper is designed to provide preliminary ethical considerations for various stakeholders, based on lessons learned from the emerging ethical discourse concerning genome editing. CRISPR has accelerated historical debates and reforms and may exacerbate existing technological disparities and threats of technological misuse (D'Souza *et al.*, 2024; Subica, 2023). We suggest broader engagement with questions concerning the ethical, legal, and societal implications of CRISPR-based scientific and technological advances.

## 8. Conclusion and Implications for Research:

In the era of increasing concern about the overconsumption of natural resources and the demand for food and health applications, the applications of CRISPR have been extended to multiple fields of life. CRISPR has made it more feasible to break through the barriers of genetic engineering applied in agriculture, alleviating great contradictions against environmental sustainability. In medicine, there is a clear argument that progress in researching CRISPR technology has contributed substantially to accelerating drug discovery over the years as well as having wide-ranging effects against a variety of viruses, including those in respiratory diseases. There is a growing body of research that continues to develop and improve gene editing techniques through mechanisms and structures of CRISPR systems. Until now, studies have been very concerned with scaffolding molecules for Cas protein-guided DNA targeting, modifying complexes, and other innovations that enhance the effectiveness of Cas proteins.

This gene modification tool has proved marvelous and has sensitively interfered with a very vast area of study in various branches of sciences. From the above information, the following conclusions can be made: The research on the mechanisms of bacterial immunity that has been conducted for the first time caused applications of various gene editing tools capable of inducing the use of bacteriophage immunity for the life fields in medicine, biotechnology to create a healthy sustainable environment. The usefulness of this invention overshadows the chances of wrong usage of the found technology. Although the mentioned aspects are the weaker parts of this field for researchers to expand and enhance the remaining lacunas within this context through policies and ethical frameworks to safely use CRISPR for humans, animals, and the sustainable use of ecosystem and natural resources. Further research in this area should focus on augmenting scientific invention through bioinformatics of CRISPR associated sequences. Other findings extend to predicting the ability of the bacterial community in modulating the immunomodulation of eukaryotic cells.

### 8.1. Summary of Key Findings:

The clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (Cas) systems are RNA-guided anti-virus and anti-plasmid immune systems found in prokaryotes. Ever since the demonstration of the purified Cas9 enzyme's ability to introduce a double-strand break in a desired DNA site using guide RNA, the use of CRISPR for configurable genome editing has increased dramatically. Here we reviewed the mechanisms of the CRISPR-Cas systems, the main components used for bacterial immunity, and the relevant types of CRISPR-Cas. We also delved into the applications of these systems, which include gene editing, gene activation and repression, nucleic acid detection, and RNA tracking. The parameters determining the specificity and efficiency of the Cas and its application as an antimicrobial against pathogenic bacteria were also discussed.

CRISPR, RNA guiding, and the various Cas systems have been reviewed. The main findings from this review include:

- The CRISPR-Cas systems are bacterial and archaeal immune systems with three main mechanisms.
- There is a myriad of Cas proteins, guide RNAs, and PAMs evolved for bacterial immunity against invading phages.
- The type I, type II, and type V systems are the most studied, while the newer superfamilies II and VI are receiving attention for their applications, including gene editing, gene expression regulation, target RNA cleavage,

nucleic acid detection, and basic research. Despite the plethora of Cas families, only a few Cas families are employed for these applications.

- Some tools provide faster and simpler applications than others.
- CRISPR can be used as potential antibiotics to treat antibiotic-resistant bacteria, human immunodeficiency viruses, cancer, and amyotrophic lateral sclerosis. There is much research still to be done to clarify the mechanism and improve the applications of CRISPR-Cas systems. CRISPR technology has shown groundbreaking applications in a wide range of fields such as organisms and diseases. However, if the challenges—technical and ethical—are not addressed, the impact would be far from mainstream. Although much progress has been made, further studies are required for better mechanistic insight and enhanced tools. In particular, improved applications of type I and II systems are anticipated.

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