Widespread Foreign Protection: CRISPR/Cas and Other Prokaryotic Immune Defense Systems

Samika Arun[1] and Arianna Broad[2]

[1]Mountain View High School

[2]Cornell University

Abstract:

CRISPR/Cas - clustered regularly interspaced palindromic repeats and CRISPR associated proteins – form a complex in prokaryotes used to combat foreign invaders. Despite its efficacy, this immune defense system is often employed alongside others to provide maximum protection for prokaryotes. The scope of this paper will review which defense systems are present in different subcategories of prokaryotes and when each one is employed during foreign invasion.

Introduction:

In 2012, the discovery of the CRISPR/Cas9 system in prokaryotes revolutionized the world of gene editing. The ability to store foreign viral DNA within palindromic repeats of nucleotide sequences combined with the Cas9 enzyme's ability to cut strands of DNA at said specific sequences from the foreign DNA has enabled scientists to manipulate the CRISPR/Cas machinery for the alteration and repair of DNA mutations within eukaryotes, focused on palliative treatments for genetic conditions. The number of studies surrounding the application of the CRISPR/Cas complex in eukaryotes has exponentially increased since its specific abilities were revealed. However, it originally functioned not as a system to isolate mutations in DNA, but as an adaptive immune defense system in bacteria and archaea to protect them against bacteriophage invasions. Its prevalence certainly proves its efficacy, as CRISPR cassettes, the complete components for CRISPR function, are found in 90% of archaea and 40% of bacteria (1). This high prevalence is likely due to the horizontal transfer of traits between bacteria and archaea respectively (1). However, this leaves roughly 60% of bacteria without this highly effective immunity. It is thought that the lack of a CRISPR/Cas system has encouraged the evolution of alternative immune systems, both adaptive and general, in the remaining prokaryotes. Some prokaryotes may have alternate defense systems in addition to CRISPR immunity, which are employed in response to different environmental stresses.

CRISPR immunity and its flaws

CRISPR is an adaptive-immune defense system that generally functions through 3 steps – the acquisition and integration of foreign DNA between clustered, regularly interspaced short palindromic repeats (CRISPR) present in the host genome, the expression of this CRISPR locus as pre-crRNA, and the use of this RNA to guide the Cas9 protein to the corresponding foreign locus to subsequently degrade the foreign invading DNA (2, 3). However, despite the likely high efficacy rate implied by the high prevalence of CRISPR immunity systems (1), the rapid rate of mutations in the nucleic acid sequences of phages allows them to compete with CRISPR/Cas immunity to avoid degradation. This can include single nucleotide point mutations that prevent recognition and subsequent binding of the complex (4). Additionally, more complex phages can produce anti-CRISPR (Acr) proteins that block the binding or cleavage steps of CRISPR immunity (4). Due to these methods employed by phages to avoid the specific, adaptive CRISPR immunity, many bacterial lineages possess other types of immunity that are used

during invasion, simultaneously or subsequently to the formation and operation of CRISPR/Cas. These defensive immune systems differ vastly based on the environmental conditions and identity of each type of bacteria.

Types of Common Immune Defense Systems in Bacteria:

Restriction Modification (RM) System

One general defensive immune system present in almost all prokaryotes is the restriction-modification system (5). Furthermore, it is often present in bacteria additionally to CRISPR. The RM system functions by DNA methyltransferase selectively identifying a nucleotide sequence in the host genome and adding a methyl group to a specific nucleotide matching that of the genome of a phage. DNA endodeoxyribonuclease then recognizes and cleaves the targeted sequence in the phage genome, while the host DNA is protected (6). One study showed that in *Streptococcus Thermophilus*, a type I RM system present in the bacteria acted alongside CRISPR/Cas systems, both working to cleave foreign DNA to prevent further invasion (a). Additionally, the methylation of host DNA caused by the RM system did not interfere with the productivity of the Crispr/Cas system (a). While this general immune response is abundantly found in prokaryotes, it is important to note that it is not found in obligate symbionts (5).

Toxin-Antitoxin (TA) System

The toxin-antitoxin (TA) system is a highly abundant, (7) general immune response (8) composed of two genes with nucleotide sequences coding for a toxin and neutralizing antitoxin (7). When the bacteria experiences environmental stress, the stabilizing antitoxin degrades, and the toxin hijacks the chromosomal machinery within the bacteria to inhibit phage replication and propagation (8). An important distinction for this immune system is that the toxin coded by the TA system reduces the metabolism of the bacteria but does not cause programmed cell death. Though TA systems are general immune responses, recent studies have shown that the highly specific CRISPR/Cas systems originally evolved from TA systems through random mutations (8). These random mutations in the TA system leading to the evolution of the CRISPR/Cas system may explain why the CRISPR/Cas system currently focuses on degrading and inhibiting the foreign invader instead of modulating the host transcription/translation machinery.

Abortive Infection (Abi) System

The abortive infection system found in many prokaryotes differs from TA systems in that it does not aim to preserve the prokaryote and instead incites its death in an altruistic move (9). The types of abortive infection systems differ greatly due to their ubiquity, however, all ABi systems contain one component that recognizes a phage infection and one that kills the bacteria, usually by shutting down protein production machinery (10). Since ABi systems induce cell death, they are essentially not a self-defense mechanism in bacteria but a way to prevent the phage invasion from spreading, like quorum sensing (11). It is a system employed after all other defense immune systems fail in the bacteria, so it usually only occurs if the CRISPR/Cas system is not successful in degrading the phage genome. However, recent studies have suggested that some CRISPR/Cas system types can ultimately lead to abortive infection (12).

Prokaryotic Argonautes (pAgos)

In both eukaryotes and prokaryotes, Argonaute proteins are involved in RNA interference (RNAi), used to silence genes (13). Unique to prokaryotes, however, is the ability for prokaryotic Argonaute proteins (pAgos) to employ guide DNA or RNA to cleave the DNA or mRNA of a phage (14). With so many other immune defense systems present in eukaryotes, pAgos are relatively less involved during an invasion, which explains their lower prevalence rate of about 40% in prokaryotic genomes (15). Since the Ago gene is constitutively expressed in some prokaryotes, pAgos are likely a part of the initial immune response in prokaryotes (15). While pAgos are considered less effective than CRISPR due to their lack of memory of each invasion, recent studies have shown that the cleaved DNA produced by pAgos may be acquired by CRISPR in between spacers, leading to a new line of questioning to study in the future (15, 16).

Classes of Bacteria and their Immune Defense Systems

Obligate Symbionts

Obligate symbiotic bacteria rely on their hosts for resources such as nutrients and/or a stable living environment. This heavy dependence encourages an interconnected nature between the host immune system and the bacterial immune system – one that can be exploitative or cooperative and is usually unique to the host. If exploitative, the symbiotic bacteria may interact with the host by suppressing its immune system in order to allow further propagation and density of the bacteria (17). This can occur through altering the expression of central immune-related genes, which is what the bacterial species *Regiella insectiola* carries out in its pea aphid host (17). Conversely, if the obligate symbiont is cooperative, the bacteria may enhance the host immunity during a bacteriophage invasion. For example, the symbiotic bacteria *Protochlamydia amoebophilia* that lives in amoeba hosts provides a shorter recovery period for the amoeba after infection by the pathogenic *Legionella pneumophila* (18). Upon infection, multiple genes related to environmental stress are expressed at a higher rate in *P. amoebophila*, and the proteins created by these genes cooperatively interact with the host amoeba to decrease the infectivity of L. pneumophila, protecting both *P. Amoebopila* and its host. (18)

Extremophiles

The category of bacteria classified as extremophiles is extremely broad, as it describes all prokaryotes that live in habitats that are unhospitable for humans and most eukaryotic life (19). Since they live in extreme environments and are often consequently exposed to constant environmental stressors, many extremophiles have developed unique immune systems or have significant differences in the immune systems they share with other bacteria. For example, genome sequencing of the thermophilic *Thermosipho* genus revealed a high abundance of CRISPR/Cas systems but sparse number of functional RM systems in the bacteria (20). This is likely explained by the need for a specific defense mechanism like CRISPR, over a general system needed when a bacterium constantly faces infections as a result of the extreme hydrothermal environment the *Thermsipho* genus bacteria are found in.

Escherichia coli (laboratory bacterial strains)

Escherichia coli is a common bacteria found in the intestines of humans and animals that has been extensively studied due to its genetic simplicity, rapid growth, and genetic malleability (21). Some of the immune systems that have been studied in E. coli and were detailed above include two independent and

unique CRISPR/CAS systems (22), a TA system encoding the toxin MazF neutralized by the MazE antitoxin (23), a Rex ABi system (10), and multiple restriction modification module systems (24, 25), among numerous others. This high diversity of immune systems in Escherichia coli can most likely be explained by its generally stable and neutral environment, as well as the prevalence of the bacteria.

Even though E. coli has been extensively studied regarding immune defense, it is imperative to continue to study E. coli for several reasons. The first and most important reason is due to its quotidian use for molecular biology, genetics, biochemistry, and more. Many proteins can be expressed using E. coli, however, some proteins cannot be expressed. It is frequently unknown why certain proteins won't express in E. coli, but it is hypothesized that this could be due to upregulation of proteasome function upon transformation and expression of foreign DNA. This problem in turn could be solved by studying E. coli's immune defense systems.

Discussion

The CRISPR/Cas system is a highly effective defense system in prokaryotes that is employed against phages, and other foreign DNA. However, this form of immunity has limitations which many phages are able to exploit. This leads to a mechanism by which the CRISPR/Cas system can be enhanced or replaced by other defense mechanisms. It is thought that one of the first line of defenses during an invasion are prokaryotic Argonaute proteins, due to their constitutive expression of these proteins that can cut the genome of foreign invaders. An example of synergistic deployment of immune defense systems is the usage of Restriction Modification and CRISPR/Cas systems as defense. Both defense systems are extremely widespread in prokaryotes that often occur simultaneously to enhance protection of the host genome and degradation of the foreign genome. The last line of defense for prokaryotes are the Toxin-Antitoxin and Abortive Infections Systems. These systems act as mechanisms to avoid further propagation of the virus and are subsequently employed closer to the crux of an invasion. The Abortive Infection system is the very last system that can be implemented by a prokaryote, as it causes cell death in an altruistic attempt to protect nearby prokaryotes.

Many of these immune systems can be found in most of all studied archaea and bacteria, however the intricacies of each system vary extensively based on the environmental conditions that the prokaryote is exposed to or cohabitates in. For example, if a bacterium relies on its host for nutrients to survive, its mechanisms for defense will be highly interconnected with that of is host organism. This is because the bacterium's survival is dependent on its host, so to ensure its own protection, it must first ensure the protection and survival of the host, this is what we call obligate symbionts. Similar to this adaptation found in obligate symbiote bacteria, prokaryotes living in extreme environments have adapted to express immune defense genes that enhance their survival. One genus of bacteria that demonstrate this phenomenon are the *Thermsipho* bacteria found in extreme hydrothermal environment. These bacteria have multiple CRISPR/Cas systems but very few RM systems because of their need for a specific defense system, caused by their isolated surroundings. Finally, common bacteria such as E. coli have adapted to express multiple defense genes, as their stable environments are suitable for many phages, meaning these bacteria constantly face infection and therefore require both general and specific defense systems to effectively combat all types of phages. These defenses include the CRISPR/Cas system, Restriction Modification system, Toxin-antitoxin system, Abortive Infections system, Prokaryotic Argonautes, and more, working simultaneously to create an umbrella of protection ready for an invasion.

Methods

Literature curation was completed by searching for these key terms (x,y,z) in the National Center for Biotechnology Information (NCBI) database. Out of x results, y papers were analyzed to synthesize this literature review.

Acknowledgements

I would like to acknowledge Lumiere Education for funding this research. I would like to also acknowledge Arianna Broad from the Weill Institute of Cellular and Molecular Biology at Cornell University for her guidance in my literature curation, writing, and figure generation.

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