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Anti-CRISPR: Phage's Retaliation Discloses the New Defense Plan

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Abstract:

Battle for survival between viruses and bacteria has been continuing for a long time by constantly defeating one another through their different molecular mechanisms. Bacteria use Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), as a defense mechanism, which performs its activity via Cas enzyme against phages to destroy their inheritance material, i.e., nucleic acids. However, in contrast, phages develop a new arsenal against bacterial attack, named Anti-CRISPR (Acr), that blocks the CRISPR complex. Using an anti-CRIPR mechanism, phages deceive and defeat bacteria by inhibiting binding of CRISPR complex to its original target. As far as molecular applications are concerned, CRISPR and Anti-CRISPR systems are relating to each other in various ways. From defensive approach to molecular plans, both systems are somehow collaborating in multiple ways. This review article is focusing on presenting the development of new phage's defense and molecular plans of CRISPR and Acr system in precise form. Discovery of new defense system in viruses is good for them, but lot of research is required to identify how multiple genes are evolved against CRISPR.

INTRODUCTION

Bacteria and viruses are in an endless war for survival for years and do not seem to end easily (Seed, 2015). However, bacteria are still sustaining their presence against viral attacks via their adapted defense mechanisms which help in destroying invaded viruses (Breitbart and Rohwer, 2005; Krüger and Bickle, 1983). Towards this direction, bacteria have evolved different defense mechanisms such as; restriction modification system, superinfection exclusion, and abortive infection mechanism against viruses. In addition, bacteria have evolved new mechanism named CRISPR that protects bacteria from viral infection by using sequence memory that serve as history of infection. CRISPR-Cas termed as Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated gene, classification of which are based on the incorporation of phage particle into the CRISPR locus and the way of expression of system against invaders (Makarova *et al.,* 2011). This system works on the principle of RNA mediated nucleases in which CRISPR-RNA (crRNA) guides for nuclease activity (Garneau *et al*., 2010; Westra *et al.,* 2012). Fifty percent of sequenced prokaryotes have the CRISPR system (Makarova *et al.,* 2015). CRISPR-Cas system activates upon the entrance of phage particle into the bacterial cell which leads to the fragmentation of foreign genetic material and leads to the incorporation of fragmented genetic materials into the CRISPR locus as a memory sequence for next encounter of same phage particles (Barrangou *et al.,* 2007). Two major classes of CRISPR-Cas systems are prominent having six types in which it is classified. Type I, III and IV are in Class 1 system in which Cas proteins are grouped together for action while single protein performs its action in Class 2 having Type II, V and VI (Koonin *et al.,* 2017; Makarova *et al.,* 2015).

In spite of CRISPR immunity, some studies revealed the coexistence of foreign phages and bacteria for long duration and this can be achieved by simple approach of point mutation in phage (Heilmann *et al.,* 2012; Semenova *et al.,* 2011; Sun *et al.,* 2016). By this mutation, phage can even escape itself from RNA mediated nuclease system, for that reason bacteria get deceived and cannot degrade the viral genome (Fineran *et al.,* 2014). It has been seen that for long time, bacteria and viruses are in competition to protect themselves.

Disclosure about phage immunity

In 2013, the first counterattack mechanism of phage was observed in *Pseudomonas aeruginosa (P. aeruginosa)*. Although, *P. aeruginosa* have active I-F CRISPR-Cas system that can easily attack on the phages having spacer sequences which can easily be determined by this host system, but new counter attack defense system has been discovered that can infect the host and propagate in it (Bondy-Denomy *et al.,* 2013). Upon investigation, based on sequence analysis, gene sequences which are responsible for counter of CRISPR are discovered that help to make phage defensive against bacteria. Five defined proteins named AcrF1, AcrF2, AcrF3, AcrF4 and AcrF5 are involved in paralyzing host I-F CRISPR-Cas system. New study revealed that four new protein families are discovered, nominated as AcrE1, AcrE2, AcrE3 and AcrE4, that are involved in the inhibition of I-E CRISPR-Cas system of *P. aeruginosa* host (Pawluk *et al.,* 2014). Neither these proteins disturb the Cas gene expression nor affect the formation of crRNA fragments, instead they are hypothesized to be directly involved in the blockage of CRISPR-Cas complex by mimicking. So, for nine anti-CRISPR protein families have been identified with diverse sequence similarities among member proteins. In addition, phages use another mechanism of protection, in which phage deceives bacteria by using tricky approach of mutation in its PAM sequence that lead to phages protection in their host cells (Bondy-Denomy *et al.,* 2013) as shown in Figure 1. Along with anti-CRISPR proteins, phage also encodes anti-CRISPR associated 1 protein, encoded by *aca1 gene* having helix-turn-helix pattern at downstream of anti-CRISPR gene, whose main function is currently unknown. However, it is assumed that this protein may be involved in transcription regulation. It is reported that anti-CRISPR associated 1 protein coding

gene, *aca1*, is absent in those phages in which anti-CRISPR genes are absent (Pawluk *et al.,* 2016a).

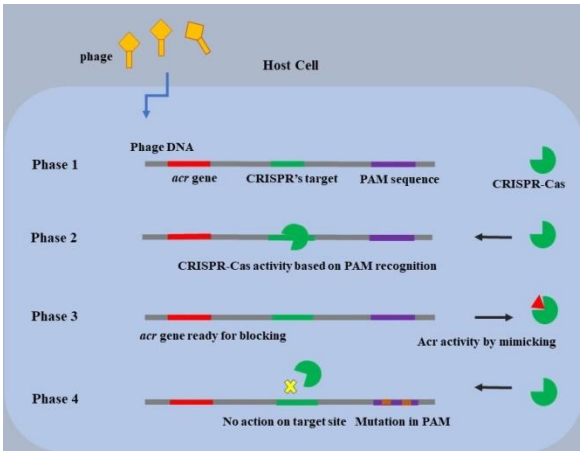

Fig. 1. Activities of CRISPR and Anti-CRISPR system are shown in multiple phases in host cell. Phase 1 representing the phage's DNA entered into the host cell, having *Acr* gene, CRISPR's target and PAM sequences site. CRISPR-Cas system of host targets in the phage DNA based on the recognition of PAM sequence in phage for its own defense against invader is showing in phase 2. While phase 3 shows that how Acr system of phage protects its DNA by producing *Acr* gene which binds to the CRISPR-Cas and blocks it that makes CRISPR-Cas unable to bind to its target. Similarly, following Acr system, phage also defends itself from host arsenal system by dodging CRISPR-Cas via causing mutation in phage's own PAM sequence, by which CRISPR-Cas system is unable to recognize PAM sequence, and cannot act on its target, resulting in protection of phage.

Mechanism of defensive molecular strategy of Phages

Generally, I-F CRISPR-Cas system is executed in three steps, first is identification, followed by cleavage and then addition of new targeted sequences in CRISPR array by Cas1 and Cas2 protein. Upon arrival of new phage into the bacterial genome, CRISPR array transcribe premature CRISPR-RNA which is then cleaved to form mature CrRNA, aided by Csy4 protein, bound to the 3´end of CrRNA. The Csy4, an endonuclease protein, combine with CrRNA to form CrRNA-Csy4 complex (Haurwitz *et al.,* 2010). Finally, Csy complex is formed by the interaction of different proteins such as: Csy1, Csy2 and Csy3 with CrRNA-Csy4 protein complex (Wiedenheft *et al.,* 2011). This Csy complex analyzes the whole bacterial cell for invading phage DNA to target, upon identification, this mature CrRNA binds to the phage's DNA, and Csy complex hires the Cas9 protein for degradation of targeted DNA (Huo *et al.,* 2014; Westra *et al.,* 2012). The 5´end of CrRNA is occupied by Csy1-Csy2 heterodimer, while 3´end of crRNA is covered by Csy4 protein and RNA spacer are covered by six different subunits of Csy3 protein in Csy complex (Haurwitz et al., 2010; van Duijn et al., 2012). However, three anti-CRISPR proteins i.e., AcrF1, AcrF2 and AcrF3 play key role in inhibition of CRISPR mechanism process and give strength to phage to make it defensive against bacterial CRISPR system. Attachment of these AcrFs proteins to the Csy complex, activates the phage defense system. Among these three Acr proteins, AcrF1 and AcrF2 attaching directly to the Csy complex resulted in blockage of different sites of CrRNA, which hijacks it to perform its defensive function. Prior to phage's DNA being targeted, interaction of AcrF1 and AcrF2 proteins take place with Csy complex resulting in blockage of binding of DNA. Molecular investigation shows that AcrF2 protein binds with the Csy1-Csy2 heterodimer, resulting in the blockage of 5´ end of CrRNA, while AcrF1 binds with the subunits of Csy3 protein, the one which covered RNA spacer. On the other hand, AcrF3 which is the third anti-CRISPR protein, blocks the availability of Cas3 enzyme to the Csy complex. By the involvement of all these anti-CRISPR protein, CRISPR-Cas system switches off. It was found that AcrF1 and AcrF2 protein work in allosteric mechanism (Maxwell *et al.,* 2016). Although some Acr proteins are identified but knowing the evolution of Acr genes are still highly challenging as they found in variations and in mutated form (Li *et al.,* 2022).

CRISPR and Anti-CRISPR on different molecular plans

Anti-CRISPR came on a scene with new game plan by regulating, affecting, and controlling the CRISPR activity in various ways. Lot of applications of both systems are proposed and being discovered in series of researches. Some of them are described below that how CRISPR and Anti-CRISPR are dealing with different molecular phenomenon.

Phage Therapy

Antibiotic resistance in bacteria is one of the major problems and bacteriophage came up as an alternative of antibiotics. Bacteriophages are being used against pathogenic bacteria since many years and are known as phage therapy. (Salmond *et al.,* 2015; Wittebole *et al.,* 2014). In addition, study revealed that usage of phages containing engineered Acr proteins can be the viable strategy to patients having bacterial infection (Qin *et al.,* 2022).

Emergence of antibiotic resistance in bacteria and low rate of new drug discovery led to the usage of the phage based therapies (Domingo-Calap *et al.,* 2018; Lu *et al.,* 2011; Viertel *et al.,* 2014). Schooley *et al.,* (2017) reported in their study that one of the patient who got infected by multi-drug resistant *Acinetobacter baumannii* strain during travelling to Egypt, remained in coma for 2 months until he got an intravenous injection of a phage cocktail which lysed the bacteria and patient recovered completely after 2 days of this treatment. The development of CRISPR system in bacteria for its protection from phage is also becoming a hurdle in the phage-based therapies. This therapy is halted by CRISPR-Cas9 technologies derived from type II CRISPR-Cas adaptive immune systems of bacteria in order to target and destroy foreign DNA entities such as bacteriophages and plasmids (Barrangou *et al.,* 2007; Deltcheva *et al.,* 2011). On the other hand, discovery of Acr system is supportive for phage therapy as it is considered as an alternative to antibiotics. But CRISPR-Cas systems in some pathogenic bacteria such as *P. aeruginosa* and *Clostridioides* stop the phage propagation and lysis in host bacterial cells (Boudry *et al.,* 2015; Debarbieux *et al.,* 2010; Deltcheva *et al.,* 2011) and this limitation of phage therapy can be overcome by engineered phages containing Acrs (Saha and Mukherjee, 2019; Stanley and Maxwell, 2018).

Gene drive system

The limitation of this approach is that once gene drive gets entered in the environment, it will be difficult to remove it and it could lead to the unpredictable ecological consequences. On the basis of such expected results and other considerations, it is needed to improve the performance and safety of CRISPR-Cas9 applications by controlling its Cas9 activity. Many groups have developed methods for providing specific cues i.e. light-inducible and drug-inducible Cas9 activity in order to activate CRISPR-Cas genome editing activity (Nihongaki *et al.,* 2015; Nuñez *et al.,* 2016; Wright *et al.,* 2015). While Acrs such as type II-A or type II-C Acr proteins can be used to inhibit the Cas9-based gene drive system which is used for eradicating disease vectors such as mosquitos over a long time frame (Hammond *et al.,* 2016). In this way, Acr proteins can prevent the unpredictable ecological changes occurrence by Cas9-based gene drive system via switching off the CRISPR-Cas systems. Recent report reveals that AcrIIA2 and AcrIIA4 proteins can be used for inhibition of active gene drive systems in budding yeast (Basgall *et al.,* 2018).

Off-target effects

Off target effects is one of the main challenges for researchers and it is being resolved by several approaches. To restrict the actions of CRISPR-Cas tool to target is the main hurdle in genome editing. And this is because of Cas nuclease activity, as long as it remains with guided RNA, which guides for nuclease, it keeps doing its activity even as it surpasses its original target which lead towards off-target mutations. Anti-CRISPR can be the solution to this, in such a way that it can be used to limit Cas enzyme's nonspecific activity (van Houte *et al.,* 2016). The undesired off-target effects are occurred due to the excessive or prolonged Cas9 activity of

CRISPR. This problem can be solved by bringing modifications in Cas enzyme, while to overcome this problem, sgRNA is designed to increase the specificity of target recognition (Kleinstiver *et al.,* 2016; Kocak *et al.,* 2019). The discovery of Acr proteins also opened the doors to limit the Cas activity at the target sites (Pawluk *et al.,* 2016b; Rauch *et al.,* 2017). As it is reported that AcrIIA4 reduced off-target effects through inhibition of Cas9 activity in a timely manner in cells (Shin *et al.,* 2017). Similarly, type IIA, type IIC, and type VA Acrs can inhibit Cas9 or Cpf1 based genome editing in human cell lines (Hynes *et al.,* 2018; Lee *et al.,* 2018; Pawluk *et al.,* 2016b; Shin *et al.,* 2017; Watters *et al.,* 2018). Recently, a research indicates that timed addition of AcrIIA4 can lessen the undesired off-target effects in human cells (Shine *et al.,* 2017) that show its clinical potential for applications in future.

CONCLUSION

Bacteria and viruses remain in battle over many years and are still evolving new defensive molecular strategies against each other. While using as genome editing tool, CRISPR is the more accurate and fast technique than previous ones. Although trials are not yet practicing in humans due to ethical considerations, it seems that this technique would be successful in near future. On the contrary to CRISPR, new arsenal has been discovered in phages named anti-CRISPR. Investigations are ongoing to explore more about anti-CRISPR mechanism. There is no doubt that it opened the new doors to study more about phage defense system. It will be a startling discovery that how the virus will influence and aid in the genome editing to improve this technique.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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