The CRISPR Counterattack: Anti-CRISPR Proteins

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The CRISPR-Cas system has revolutionized genome editing, enabling precise modifications and holding promise for groundbreaking discoveries and therapies. However, significant challenges persist, including off-target effects, cellular toxicity, and immunogenicity, which must be addressed to ensure reliable and safe applications. Interestingly, bacteriophages have evolved Anti-CRISPR proteins to counteract bacterial CRISPR-Cas immunity, allowing them to infect and survive. These proteins have proven to be the most effective strategy for phage survival, outsmarting bacterial defenses that adapt through "priming adaptation." As the CRISPR-Cas toolbox expands, understanding and harnessing Anti-CRISPR proteins may provide crucial insights for improving genome editing technologies and overcoming existing challenges, ultimately unlocking the full potential of CRISPR-Cas for molecular, biotechnological, and medical innovations.

History of Anti-CRISPR proteins

According to Brouns's Red Queen hypothesis, species must continually evolve new resistance mechanisms to avoid extinction, as parasites adapt and counter-adapt in an ongoing evolutionary arms race. This dynamic is exemplified in the co-evolutionary interplay between bacterial populations and bacteriophages, where phages have developed Anti-CRISPR proteins to evade bacterial CRISPR-Cas defences. The discovery of Anti-CRISPR systems began with *Pseudomonas aeruginosa* prophages, which disabled type I-F CRISPR-Cas systems. Researchers identified nine Anti-CRISPR proteins (AcrF1-5 and AcrE1-4), targeting CRISPR-Cas interference. Anti-CRISPR proteins work together with transcriptional regulators (Aca1 and Aca2). Five new type I-F anti-CRISPR proteins (AcrF6-10) were discovered. Twenty-one Anti-CRISPR protein families have been discovered. Ongoing research aims to uncover more due to phages' rapid evolution.

Structure of Anti-CRISPR proteins

AcrIIA4 is a protein consisting of 87 amino acids with a molecular weight of 10.182 kDa. Its structure comprises flat sections (β-strands), spiral sections (α-helices) and connecting loops, with a notable hydrophobic core featuring aromatic clusters. Additionally, AcrIIA4 has a high concentration of negatively charged residues, particularly in areas between its flat and spiral sections. These negatively charged residues may play a crucial role in inhibiting Cas9 activity by mimicking nucleic acid phosphates.

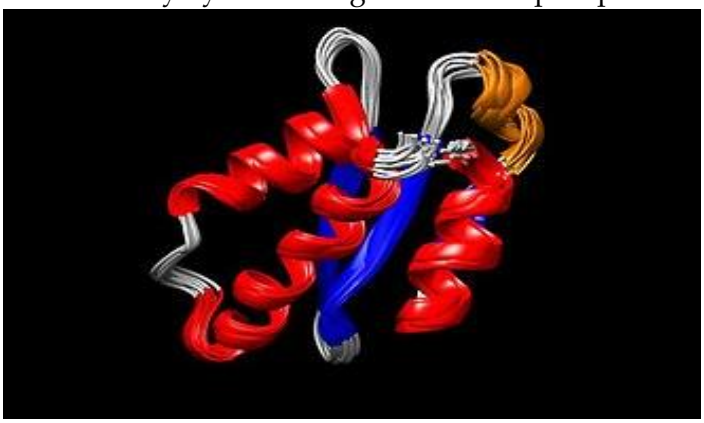

Fig. 1. Structure of AcrIIA4 obtained with the UCSF Chimera software, Different colours were assigned to the four different secondary structures found in this protein: blue for β-strands, red for α-helices, orange for the 3_{10} helix, and grey for loops (Kim *et al*., 2018).

Functions

Protecting Phage DNA from Destruction

Anti-CRISPR proteins play a crucial role in protecting phage DNA from destruction by the host's CRISPR-Cas immune system. Here's how

1. Phage DNA enters a prokaryotic cell.
2. The cell detects the target sequence, activating CRISPR-Cas immunity.
3. Initial sequences encoding Acr proteins are read before the target sequence.
4. Acr proteins form, blocking CRISPR-Cas response.
5. CRISPR locus transcription yields crRNAs, combining with Cas proteins to form Cascade.
6. Cascade searches for complementary sequences and recruits Cas3 nuclease.
7. AcrF1, AcrF2, and AcrF3 interact with Cas proteins, preventing phage DNA binding and cleavage.

Additionally, Aca genes, which encode proteins with a helix-turn-helix DNA-binding motif, regulate Acr transcription, ensuring phage survival by repressing constant transcription.

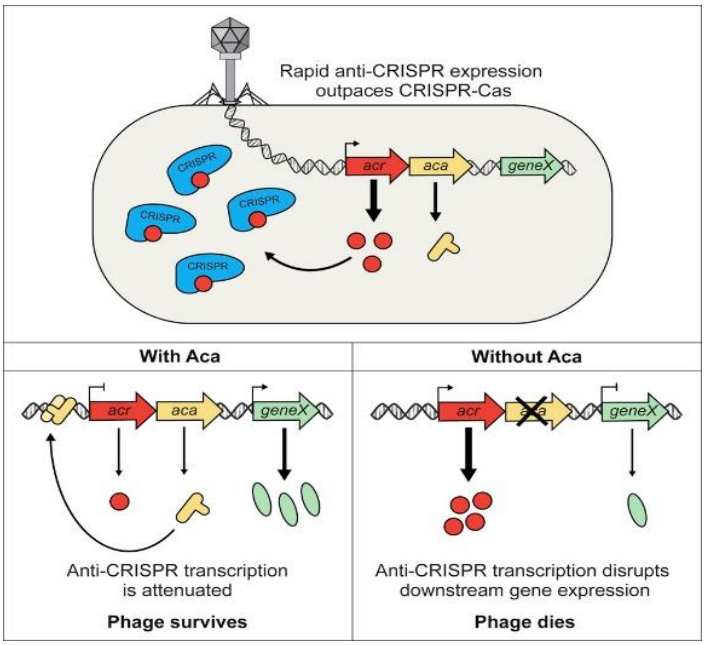

Fig. 2. Protecting phage DNA: Phage DNA enters a prokaryotic cell, triggering CRISPR-Cas immunity, but Acr proteins encoded by upstream sequences form and interact with Cas proteins, ultimately preventing phage DNA binding and cleavage (Stanley *et al*. 2019)

Phage-phage cooperation

Bacteria with CRISPR-Cas systems retain partial immunity to Anti-CRISPR (Acr) proteins, limiting initial phage infections' ability to evade immunity. Phage-phage cooperation is a synergistic strategy where bacteriophages work together to enhance their infectious capabilities and promote epidemic spread. Through this collective effort, phages amplify Anti-CRISPR protein production, evading host immunity and suppressing host defenses. This creates an epidemiological tipping point, where the outcome depends on the initial phage density and binding strength. If the threshold is exceeded, successful infections outnumber unsuccessful ones, leading to epidemic spread and phage persistence in immunocompromised hosts. Conversely, if the threshold is not reached, phage extinction occurs. This cooperative mechanism allows phages to adapt and overcome host immunity, ultimately determining the fate of phage populations.

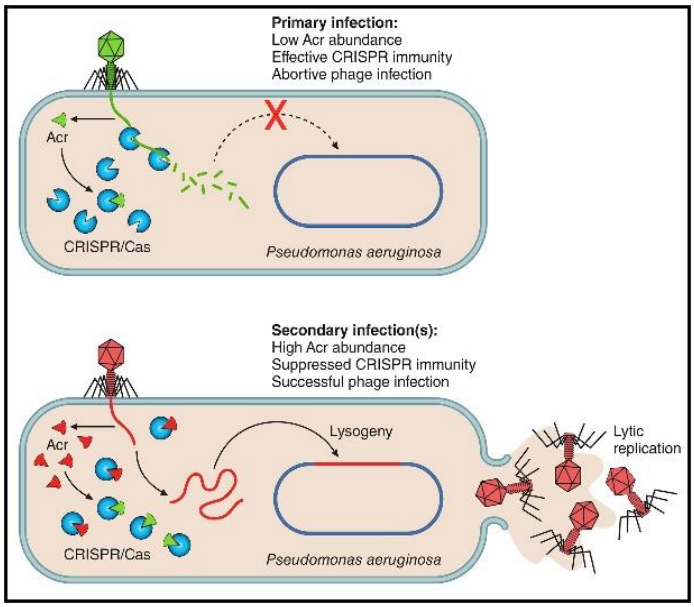

Fig. 3. Phage-phage cooperation: Initial phage infections often fail to overcome CRISPR immunity, but subsequent phage-phage cooperation significantly enhances Acr production and host immunosuppression. This cooperative effort increases the host cell's vulnerability to reinfection, ultimately facilitating successful infection and propagation of additional phages (Gent and Gack, 2018)

Phage Immune Evasion

Phage immune evasion relies heavily on Acr proteins, which enable phages to circumvent the host's CRISPR-Cas defense system. Although the exact mechanisms remain unclear, Acr proteins facilitate phage survival by counteracting the host's CRISPR-Cas system, which would otherwise rapidly degrade the phage genome following infection.

1. Binding to CRISPR-Cas components: Acr proteins interact with Cas enzymes, preventing DNA cleavage.
2. Inhibiting CRISPR-Cas assembly: Acr proteins disrupt Cascade formation, hindering target recognition.
3. Blocking CRISPR-Cas activation: Acr proteins prevent CRISPR-Cas system activation.

Mechanisms of Anti-CRISPR Proteins

CrRNA Loading Interference

In Type II CRISPR-Cas systems, such as *Streptococcus pyogenes* SF370, utilize a unique crRNA maturation pathway involving an additional trans-activating CRISPR RNA molecule (tracrRNA) and

host RNase III. During the interference step, mature crRNAs combine with Cas proteins to form an effector complex, which recognizes and binds to the target sequence in invasive nucleic acids through complementary base pairing. This binding induces sequence-specific cleavage, effectively preventing the proliferation and propagation of foreign genetic elements.

DNA Binding Blockage

Multiple Anti-CRISPR proteins, including AcrIIC2, AcrIF1, AcrIF2, AcrIF10, AcrIIC3, and AcrIIA2, can block DNA binding to the CRISPR-Cas complex. AcrIF1, AcrIF2, and AcrIF10 target different subunits of the type I-F Cascade effector complex, preventing DNA binding. Additionally, AcrIIC3 promotes Cas9 dimerization to block DNA binding, while AcrIIA2 mimics DNA, blocking PAM recognition residues and preventing dsDNA recognition and binding, thereby inhibiting CRISPR-Cas activity.

DNA Cleavage Prevention

Anti-CRISPR proteins targets Cas9 site and prevent DNA cleavage. For example, AcrE1, AcrIF3, and AcrIIC1 proteins inhibit target DNA cleavage through distinct mechanisms. AcrE1 binds to Cas3 via X-ray crystallography, blocking Cas3's association with the Cascade complex. Similarly, AcrIF3 forms a dimer to bind Cas3, preventing Cascade recruitment. Conversely, AcrIIC1 targets Cas9's active site, rendering it inactive while bound to DNA, thereby preventing cleavage.

Applications

Reducing CRISPR-Cas9 off-target cuts

Anti-CRISPR proteins inhibits the CRISPR-Cas9 system, crucial for cell editing. Adding AcrIIA4 to human cells prevents Cas9 interaction with CRISPR, reducing DNA cutting. However, studies suggest that introducing Anti-CRISPR proteins in small quantities post-genome editing minimizes off-target cuts at specific Cas9 interaction sites, significantly enhancing precision.

Aid in Synthetic biology

Another area of current study for programming unique cellular behaviors, such regulating development or incorporating biological sensors and devices is synthetic circuits that control gene expression. Anti-CRISPR proteins enable control over genetic circuits.

Prevention of biological containment

Anti-CRISPR proteins prevent genetically modified organisms from spreading. Release of organisms with Acr proteins were found to block the spread of suppressive gene drive penetrating into the population and hinders from suppression.

Conclusion

The discovery of Anti-CRISPR proteins marks a significant development in the field of genome editing. These proteins, evolved by bacteriophages to counteract bacterial CRISPR-Cas immunity, represent a natural mechanism to mitigate off-target effects, toxicity, and immunogenicity associated with CRISPR-Cas systems. By interfering with various stages of the CRISPR process—such as DNA binding, cleavage, and system activation—Anti-CRISPR proteins offer a means to fine-tune genome-editing technologies.

As research continues, the integration of Anti-CRISPR proteins into existing CRISPR-Cas systems holds promise for safer and more precise genome editing, paving the way for transformative advancements in medicine, agriculture, and synthetic biology. The ongoing exploration of their mechanisms and applications may unlock the full potential of this revolutionary technology.

References

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