Proceedings of ICBioMed 2025 Symposium: Computational Modelling and Simulation for Biology and Medicine
DOI: 10.54254/2753-8818/2025.LD25615

Research on Anti-CRISPR Applications in Gene Editing, Biotechnology and Medicine

Qimin Luo

School of Life Sciences, Xiamen University, Xiamen, China
13365907705@163.com

Abstract. CRISPR-Cas systems serve as adaptive immune mechanisms in prokaryotes, while mobile genetic elements like phages have evolved anti-CRISPR proteins (Acrs) to evade this defense. This review synthesizes the principles, mechanisms, and diverse applications of Acrs, highlighting their roles in precision gene editing, gene drive regulation, phage therapy, and optogenetics. Acrs inhibit CRISPR-Cas through multiple mechanisms, including direct binding to Cas nuclease active sites, spatial occlusion of nucleic acid binding sites, induction of conformational changes in Cas complexes, and enzymatic degradation of CRISPR components, which enable precise control of CRISPR activity. In gene drive systems, Acrs act as safety switches to mitigate unintended genetic spread in organisms like Saccharomyces cerevisiae. For phage therapy, engineered phages effectively combat CRISPR-resistant Pseudomonas aeruginosa infections, while Acrs may also inadvertently facilitate antibiotic resistance plasmid spread in pathogens like Klebsiella pneumoniae. This review underscores Acrs as versatile regulators of CRISPR technology, balancing precision and safety across biomedical and ecological applications, while emphasizing the need for deeper mechanistic insights and translational research.

Keywords: Anti-CRISPR, CRISPR-Cas, Gene Editing, Phage Therapy, Optogenetics

1. Introduction

The CRISPR-Cas system is the most common adaptive immune mechanism in bacteria and archaea, being present in most bacteria and the vast majority of archaea. It directs the cleavage of Cas nuclease through complementary pairing of crRNA and invasive genetic material, which achieves the destruction of the invader's genetic material. Mobile genetic elements (MGE), such as plasmids and phages, have evolved multiple means of evading this mechanism, including mutations in the Primary Interval Region Adjacent Motifs (PAM), mutations in the target Primary Interval Region (PICR), and inhibition of CRISPR interference by Anti-Anti-crispr (Acrs). Acrs proteins are a pooled library of natural CRISPR-Cas antagonists encoded by a variety of MGEs that inhibit CRISPR-Cas immune function at different stages. So far, 45 non-homologous anti-CRISPR proteins have been identified with a variety of structures and inhibitory mechanisms, including histone deoxyribonucleic acid (DNA) binding, inhibition of DNA cleavage, degradation through enzymatic activity, and alteration of complex conformation. The diversity of mechanisms presented in the Acrs library is an advantage for genome editing applications, as it provides a variety of inhibition options

© 2025 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2025.LD25615

that can be tailored to specific editing strategies. For example, Acrs that permit DNA binding but block nuclease activity can provide control over target cleavage while retaining activity of the bolus structural domains that control transcription [1]. Anti-CRISPR has been widely used in research and biomedical applications, such as controlling CRISPR-Cas system gene editing to improve safety [1], as editing off-target is a key concern for CRISPR-Cas systems to focus on in practice. In addition, anti-CRISPR applications in phage therapy, optogenetics, and other fields are under development [1]. This paper describes the principles and mechanisms of Acrs through a literature review, and presents advances in the application of Acrs in the field of controlled gene editing and gene drive, especially related to phage therapy and optogenetics. Both Acrs and the CRISPR-Cas system are powerful gene editing tools, and the use of the two in conjunction with each other will advance the precision and diversity of gene editing, drug discovery, and other aspects of gene editing.

2. Acrs

2.1. CRISPR-Cas system

The CRISPR motif consists of two parts: the array containing the spacer and the CAS motif. The spacer in the array is separated by a palindromic sequence that repeats throughout the array. The role of the CRISPR-Cas system consists of the array being transcribed to produce a CRISPR RNA molecule (crRNA) precursor, processed to a mature crRNA molecule by processing Cas proteins, and bound to Cas proteins to obtain the CRISPR-Cas complex. The site on the exogenous DNA that is targeted by the spacer region is called the pre-spacer region, which is flanked by the pre-spacer region adjacent motif PAM. The CRISPR-Cas complex binds to the pre-spacer region through base complementary pairing, recognises the PAM, and mediates the cleavage and destruction of the nucleic acid. CRISPR-Cas systems are unique in the ability to adapt to invasive MGEs never encountered before and to gain immunity to these MGEs. Since they can incorporate short sequences into CRISPR arrays by excising them from these elements and integrating them as new spacers, CRISPR-Cas systems have a high degree of diversity and complexity. CRISPR-Cas as a whole can be divided into two major classes (Class 1 and Class 2), six types (Type I-VI), and over 30 subtypes. Among them, Type II and Type V systems (e.g., Cas9 and Cas12a) have received extensive attention for applications in gene editing [2].

2.2. Acrs research

For the Acrs system, phages acquired genes encoding inhibitors of the CRISPR-Cas system, called Acrs. The Acrs protein was originally discovered while studying the effects of prophages (viral genomes that integrate intact into the host bacterial genome) on Pseudomonas aeruginosa. Bondy-Denomy et al. constructed a collection of strains containing different prophages to systematically study the phenotypic effects [2]. Utilising this collection, three prophages were identified that mediated the repression of the type I CRISPR-Cas system present in the Pae strains studied. By comparing the genomes of these prophages with those of related prophages, a region potentially containing the Acrs gene was identified in eight different prophages. Ultimately, genes encoding five completely different families of Acrs proteins (AcrIF1-AcrIF5) that inhibit the I-F type system were identified [2]. Researchers have also identified multiple Acrs families (e.g., AcrIF1-AcrIF10, AcrIIA1-AcrIIA10, etc.) through a variety of genomic comparisons and bioinformatics approaches [2]. Among them, the Guilt-by-Association Approach takes advantage of the conserved nature of the ACR gene region and finds genes that may encode ACR proteins upstream of them by searching for

DOI: 10.54254/2753-8818/2025.LD25615

genes that are homologous to Aca proteins, especially the fact that downstream of ACR genes there is usually a gene encoding Aca proteins containing helix-turn-helix (HTH) DNA-binding structural domains. Exploiting the phenomenon that phages with ACR genes in the genes survive when its own CRISPR-Cas system targets its own genome sequences, and by searching bacterial genomes encoding functional CRISPR-Cas and self-targeting CRISPR spacer sequences and comparing phage genomes, Self-Targeting can identify regions of divergence and identify possible ACR proteins in the regions of divergence. This approach was used by Rauch et al. to discover AcrIIA1 and AcrIIA2, which were able to inhibit the type II-A CRISPR-Cas system of Listeria monocytogenes [2]. In addition, functional screening method is also an effective method to discover Acrs.

2.3. The mechanism of action of Acrs

The mechanism of action of Acrs can be divided into four main categories. First, Acrs achieve inhibition by directly binding to Cas functional sites, which in this case include the HNH domain and RuvC domain of Cas9. For example, the Type II-C anti-CRISPR protein AcrIIC1 was demonstrated to directly bind to the HNH structural domain of NmeCas9, interacting with its active site residues and thereby preventing cleavage of the target DNA. Some Arcs can achieve inhibition by non-competitive binding, i.e., by simultaneously binding to different regions of the Cas protein. Second, inhibition can be achieved by spatial closure of the Cas functional site, which includes mimicking the nucleic acid structure to occupy the nucleic acid binding site of the Cas protein and binding to the PAM site. The third mechanism is to inactivate or prevent the binding of Cas proteins or CRISPR-Cas complexes by inducing conformational changes. The induced conformational changes include inducing oligomerization of Cas proteins, such as AcrIIA6 and AcrVA4, which form dimers or higher-order oligomers by binding to the Cas9 and Cas12a complexes, respectively, so as to change the binding affinity of Cas proteins, affect the dynamic balance of Cas proteins, and so on. Finally, some Acr proteins can also utilize its own enzymatic activity to accomplish enzymatic cleavage of CRISPR-Cas components (including Cas proteins and crRNAs). For example, AcrVA1, a multi-turnover endonuclease, inactivates the Cas12a complex irreversibly by mimicking the PAM sequence and localizing the catalytic residues close to the crRNA, thus achieving specific cleavage of the Cas12a-crRNA spacer sequence [3].

However, there are many Acr mechanisms that have not yet been clarified. For example, the specific mechanisms of action of AcrIF4 and AcrIIA16 still need further study. As well, the dual inhibitory ability of AcrIF2 on different systems and the molecular basis behind it also need to be explored more thoroughly [3].

3. The application of Acrs

3.1. Controlled CRISPR editing

Off-targeting occurs when CRISPR nuclease cuts non-targeted sites due to incomplete complementarity between crRNA and target DNA, resulting in unwanted insertions or deletions, and sometimes chromosomal translocations. Off-targeting is an issue that needs to be focused on when the CRISPR-Cas system is used in practice. In addition, prolonged expression of viral vector-delivered Cas9 nuclease or its derivatives and single guide RNAs could result in an increased possibility of accumulating off-target edits. Therefore, it is a popular topic to use Acrs to reduce the

DOI: 10.54254/2753-8818/2025.LD25615

off-target probability and improve the safety of editing by controlling CRISPR editing. Currently, a variety of methods and applications were identified for controlling CRISPR editing by using Acrs.

Acrs can optimize safety by controlling CRISPR editing over time, where the purpose of temporal control is to control the start time as well as the duration of CRISPR effector activity. Temporal control is accomplished by controlling the timing of Acr expression through the use of inducible promoters, as well as controlling Acr activity through the fusion of degradation determinants or photosensitive structural domains to achieve delivery of Acrs at a time later than that of CRISPR effectors [1]. AcrIIA4 was first applied successfully to control the accuracy of Streptococcus pyogenes Cas9 (SpyCas9) in human K562 cells [1]. AcrIIA4, delivered at 6 hours after SpyCas9 and sgRNA transfection, reduced off-target editing of SpyCas9. In addition to increasing the accuracy of editing, time-controlled Acrs also reduce cytotoxicity generated by the continued expression of Cas proteins and crRNAs. For example, timed delivery of AcrIIA2 and AcrIIA4 controls the duration of CRISPR-Cas9 activity and reduces cytotoxicity in human hematopoietic stem cells without affecting targeted genome editing rates [4].

Additionally, Acrs can contribute to safety by controlling the spatial editing of CRISPR. The control of ACRS restricts the location of CRISPR editing within an organism, preventing CRISPR from causing unwanted or deleterious editing in certain tissues [1]. Spatial control usually entails the use of tissue-specific promoters, such as the TBG, CD4, and CK8 promoters used in mouse liver, and Cas9, which is selectively expressed in T cells and muscle tissues. However, in certain cases, such as the complexity of CNS and liver cell types, suitable tissue-specific promoters are unavailable or simply too large to be delivered, and ACRS-based approaches can provide alternative ways to modulate anti-CRISPR expression in specific cell types by using endogenous cell-type-specific microRNAs or by fusing Acr proteins to the structural domains of photosensitive proteins.

3.2. Gene drives

“Gene drives” based on CRISPR-Cas9 technology accelerate the potential for ecological engineering. By rapidly spreading specific genes or traits within a population of organisms, ‘gene drives’ can enable ‘super-Mendelian inheritance,’ whereby a gene is inherited up to 50% more frequently in descendants than it would be under natural conditions. Gene drives are often characterized by transgenic organisms with chromosomally encoded Cas9, which is programmed to target homologous regions on sister chromatids. Cas9 cleaves the homologous region on the sister chromatid, inducing the cell to repair the break through homologous recombination, thereby copying the driver gene to the damaged chromosome. Gene drives have the potential to dramatically benefit human health, including the reduction of insect-borne diseases such as malaria or dengue, the elimination of invasive species, and increased agricultural sustainability. For example, AcrIIA2 and AcrIIA4 were proved to be effective inhibitors of gene drives in Saccharomyces cerevisiae by inactivating Cas9 [5]. However, gene drives can also have unforeseen consequences or be used for non-normal purposes, so multiple robust safety measures are required before the use of gene drive technologies in the field.

4. Applications in the treatment of drug-resistant bacteria

The misuse and abuse of antibiotics has led to a wide range of resistance mechanisms developed by bacteria, and deaths due to antibiotic-resistant bacterial infections are increasing globally [6]. However, the discovery of new categories of antibiotics has been stagnant for decades and currently relies almost exclusively on modifications of the existing medicinal chemistry framework, which is

DOI: 10.54254/2753-8818/2025.LD25615

no longer able to catch up with the rate of bacterial resistance evolution. Therefore, phages with Acrs can be used as antimicrobial compounds against those Infectious and lethal pathogens. According to Vyas, due to its host specificity and natural abundance, naturally lysed phage containing anti-CRISPR proteins can effectively provide precise treatment of severe bacterial infections with minimal side effects [7]. Phage anti-CRISPR proteins may represent the only antimicrobial therapy for the treatment of COVID-19 virus and severe acute respiratory syndrome coronavirus (SARS-CoV)-associated diseases or the subsequent bacterial infections. This is because the extensive use of antibiotics in previous treatments for COVID-19 has led to an expansion in the number of antibiotic-resistant bacteria [7].

Qin et al. constructed engineered phage EATPs by inserting type I CRISPR-resistant genes (AcrIF1, AcrIF2, and AcrIF3) into the P. aeruginosa phage DMS3/DMS3m, which was used to investigate the function of EATPs in the inhibition of P. aeruginosa infection [8]. The results indicated that EATPs could effectively inhibit the growth of drug-resistant P. aeruginosa and displayed relatively high safety in both in vitro and in vivo experiments. Experiments also demonstrated that the replication of phage is intimately related to the competition between the CRISPR-Cas system and the Acr proteins. When the output of Acr protein exceeded the threshold of the CRISPR-Cas system, the phage was able to successfully replicate and inhibit bacterial growth. The study also found that the combination of EATPs and antibiotics can effectively inhibit the ingrowth of drug-resistant P. aeruginosa and reduce the emergence of antibiotic resistance, which provides a new strategy for the treatment of drug-resistant bacterial infections.

However, the Acr gene could also be an accomplice in enhancing drug resistance in pathogens. Jiang et al. reported a newly identified Acrs gene that exerts certain effects in Klebsiella pneumoniae (Kp) [9]. The majority of infections caused by Kp are due to two pathotypes, healthcare-associated multidrug-resistant (MDR) clones and population-acquired highly virulent (hv) clones. As a plasmid “collector”, Kp usually evolves to be highly virulent by acquiring unconjugated plasmids with virulence genes and to be multidrug resistant by acquiring conjugated plasmids with antibiotic resistance genes. Jiang et al. constructed a database containing 692 putative proteins from plasmids and phages of clinically resistant or highly virulent Kp strains. Through YTH screening, a new CRISPR-resistant gene was identified, which was named acrIE10, and it encodes a protein that interacts with the Cas7* subunit of the Kp I-E* CRISPR-Cas system. acrIE10 was able to significantly restore the transformation efficiency of plasmids targeted by the CRISPR-Cas system, as well as dramatically increase the splicing efficiency of drug-resistant plasmids and retain the stability of plasmids during the passaging process. Bioinformatics analysis reveals that acrIE10 is widely distributed in a variety of bacterial and phage genomes, especially in drug-resistant plasmids. It is evident that the existence of acrIE10 may accelerate the spread of drug-resistant plasmids, increasing the risk of emergence of drug-resistant and highly virulent strains, thus constituting a potential threat to public health.

5. Light control Acrs

Optogenetics provides a valuable method for spatial and temporal control of Acr function. Optogenetics was first applied to AcrIIA4 by merging the AcrIIA4 protein with the light-responsive LOV2 structural domain of Avena sativa phototropin 1 to construct a conditionally activated light-dependent anti-CRISPR protein, known as CASANOVA [1]. Without blue light, AcrIIA4-LOV2 features inhibition of SpyCas9 derived from Streptococcus pyogenes. On exposure to blue light, AcrIIA4-LOV2 changes its conformation and does not inhibit SpyCas9. CASANOVA was initially used to control insertion-deletion formation of endogenous motifs in cultured mammalian cells in a

DOI: 10.54254/2753-8818/2025.LD25615

light-dependent manner by transfection or AAV transduction of editing components. Subsequently, CASANOVA was demonstrated to have light-dependent control of dCas9-acetyltransferase and dCas9-RFP.

As the widely used CRISPR-Cas9 direct homologue, SpyCas9 results in a high off-target rate due to its large molecular weight (158 kDa) that hinders the delivery of viral vectors. To address this problem, researchers have started to consider alternative CRISPR-Cas9 immediate homologs. NmeCas9 from Neisseria meningitidis is considerably smaller than SpyCas9, while exhibiting extremely high target specificity [10]. Thus, Hoffmannet et al. created the AcrIIC3-LOV2 fusion protein by combining AcrIIC3, an inhibitor of NmeCas9, with the LOV2 blue-light sensing domain from oat (Avena sativa), on the basis of the engineering work on AcrIIA4. By systematically screening the AcrIIC3 surface site, the researchers screened two AcrIIC3-LOV2 hybrids (CN-C3 and CN-C3(G)) successfully. And through co-transfection or AAV-mediated transduction experiments in HEK293T cells, it was demonstrated that these two hybrids could effectively block NmeCas9 activity in the dark while also permitting efficient genome editing under blue light irradiation. Hoffmann et al. optimized the performance of the photocontrol system by adjusting the ratio of CN-C3(G) to NmeCas9. It was further found that the CN-C3 could reduce the off-target editing of Cas9 and enhance the specificity of gene editing. Song et al. constructed a circularly aligned AcrIIA4 (cpA4) protein and developed a robust AcrIIA4 (OPERA4) variant by combining cpA4 with the LOV2 blue light sensory domain [11]. OPERA4 variants are mighty light-dependent tools, and OPERA4 and OPERA4v8 are enabled to achieve efficient gene editing under blue-light irradiation, whereas OPERA4v8 is more effective in inhibiting SpyCas9 in the dark and exhibits better photoswitching activity.

6. Discussion

Acrs have achieved a wide range of applications in different fields, but the investigation of Acrs is still at an early stage, with numerous areas to be explored. Among those 89 types of Acrs identified at this stage, only 29 types have known mechanisms of activity, and the related research on these Acrs only focuses on the interference step, with less attention on the adaptation and expression steps of the CRISPR-Cas surveillance pathway. In particular, the extensive use of Acrs in animal models has not yet been realized, and the safety of Acrs in relevant therapeutic applications and the possibility of using in various fields need to be further confirmed [5].

Besides the natural Acrs, other CRISPR inhibitors are being explored, such as the small-molecule inhibitors of SpyCas9 and dSpyCas9, fusion of SpyCas9 with small-molecule reactive structural domains, antisense RNA oligodeoxynucleotides (asRNAs), and suppressors of small nucleic acids (SNuBs) [1]. The evolution between prokaryotes and phages may enable prokaryotes to evolve additional strategies to oppose phages, such as anti-CRISPR strategies. Future research in the field of CRISPR-Cas system and Acr may emerge with more innovations and applications in biotechnology and healthcare. The exploitation of other CRISPR inhibitors also confronts similar issues as Acrs, such as stability within the biological environment, how to adapt to the evolution of the CRISPR-Ca system, off-target effects, and so on.

7. Conclusion

This paper reviews the essential information of CRISPR-Cas system, the principle and mechanism of Acrs, and also introduces the applications of Acrs in the field of controlled gene editing and gene drive, as well as the progress related to phage therapy and optogenetics. Throughout the

DOI: 10.54254/2753-8818/2025.LD25615

development of Acrs-related technologies and applications, it can be seen that Acrs has realized a wide range of applications in different fields, yet its research in general is still at an early stage, and still has much potential for development. As a literature review, this paper exists with the shortcomings of not comprehensively covering the content, and the topic is not prominent enough. It should understand the field more comprehensively. The authors will focus on the mechanism of action of different kinds of Acrs in the subsequent studies.

References

[1] Kraus, C., Sontheimer, E.J.(2023) Applications of Anti-CRISPR Proteins in Genome Editing and Biotechnology. J. J Mol Biol, 435(13): 168120.
[2] Davidson, A.R., Lu, W.T., Stanley, S.Y., et al.(2020) Anti-CRISPRs: Protein Inhibitors of CRISPR-Cas Systems. J. Annu Rev Biochem, 89: 309-332.
[3] Belato, H.B., Lisi, G.P.(2023) The Many (Inter)faces of Anti-CRISPRs: Modulation of CRISPR-Cas Structure and Dynamics by Mechanistically Diverse Inhibitors. J. Biomolecules, 13(2): 264.
[4] Jia, N., Patel, D.J.(2021) Structure-based functional mechanisms and biotechnology applications of anti-CRISPR proteins. J. Nat Rev Mol Cell Biol, 22(8): 563-579.
[5] Marino, N.D., Pinilla-Redondo, R., Csorgo, B., et al.(2020) Anti-CRISPR protein applications: natural brakes for CRISPR-Cas technologies. J. Nat Methods, 17(5): 471-479.
[6] Selvarajan, R., Obize, C., Sibanda, T., et al.(2022) Evolution and Emergence of Antibiotic Resistance in Given Ecosystems: Possible Strategies for Addressing the Challenge of Antibiotic Resistance. J. Antibiotics (Basel), 12(1): 1128z.
[7] Vyas, P., Harish.(2022) Anti-CRISPR proteins as a therapeutic agent against drug-resistant bacteria. J. Microbiol Res, 257: 126963.
[8] Qin, S., Liu, Y., Chen, Y., et al.(2022) Engineered Bacteriophages Containing Anti-CRISPR Suppress Infection of Antibiotic-Resistant P. aeruginosa. J. Microbiology Spectrum, 10(5).
[9] Jiang, C., Yu, C., Sun, S., et al.(2024) A new anti-CRISPR gene promotes the spread of drug-resistance plasmids in Klebsiella pneumoniae. J. Nucleic Acids Res, 52(14): 8370-8384.
[10] Hoffmann, M.D., Mathony, J., Upmeier Zu Belzen, J., et al.(2021) Optogenetic control of Neisseria meningitidis Cas9 genome editing using an engineered, light-switchable anti-CRISPR protein. J. Nucleic Acids Res, 49(5): e29.
[11] Song, G., Tian, C., Li, J., et al.(2023) Rapid characterization of anti-CRISPR proteins and optogenetically engineered variants using a versatile plasmid interference system. J. Nucleic Acids Res, 51(22): 12381-12396.