Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2024 25(3):181-196
www.jzus.zju.edu.cn; www.springer.com/journal/11585
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Review

https://doi.org/10.1631/jzus.B2300101

Defense and anti-defense mechanisms of bacteria and bacteriophages

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Abstract: In the post-antibiotic era, the overuse of antimicrobials has led to a massive increase in antimicrobial resistance, leaving medical doctors few or no treatment options to fight infections caused by superbugs. The use of bacteriophages is a promising alternative to treat infections, supplementing or possibly even replacing antibiotics. Using phages for therapy is possible, since these bacterial viruses can kill bacteria specifically, causing no harm to the normal flora. However, bacteria have developed a multitude of sophisticated and complex ways to resist infection by phages, including abortive infection and the clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. Phages also can evolve and acquire new anti-defense strategies to continue predation. An in-depth exploration of both defense and anti-defense mechanisms would contribute to optimizing phage therapy, while we would also gain novel insights into the microbial world. In this paper, we summarize recent research on bacterial phage resistance and phage anti-defense mechanisms, as well as collaborative win-win systems involving both virus and host.

Key words: Bacteriophage; Phage resistance; Abortive infection; Phage therapy

1 Introduction

Bacteria and their viruses, also known as bacteriophages or phages, have coexisted for more than three billion years. As the largest biological entities on earth, phages are also key participants in the dynamic adaptation of microbial communities, which participates in and promotes many natural cycle processes, such as the global biogeochemical cycle and human health (Barr, 2017; Mutalik et al., 2020). Phages have a simple structure comprising a protective protein capsid surrounding their genetic material, DNA or RNA. They can be divided into tailless, tailed, and filamentous phages according to their morphology (Loh et al., 2019). Phages cannot move and can approach target bacteria only by Brownian motion (Manohar et al., 2020). They are obligate intracellular parasites, and generally have two parasitic life cycles. The first is the lytic cycle, which produces and releases more phages by hijacking the metabolic machinery of host bacteria. The second cycle forms a free genetic unit (episome) or integrates into the genome of the host bacterium in the form of a prophage and maintains a "dormant state." When the host bacterium is stimulated by external adverse conditions, the latent prophage will quickly leave the host bacterium to search for the next host (Quistad et al., 2017).

Although this dormant state consumes part of the energy and material of the host, it also brings advantages to the host, such as increased virulence or stronger adaptability to harsh environments. This state of "dormancy" can be considered to be a win-win cooperation between phages and host bacteria, as some phages encode additional toxins, virulence factors, or antimicrobial resistance genes (Loh et al., 2020). The lytic life cycle cannot be fully interpreted as a balance between "ebb and flow." Phages and bacteria play the roles of predators and prey, respectively, but in the long process of evolution, both are winners. Bacteria

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Received Feb. 14, 2023; Revision accepted June 24, 2023;
Crosschecked Feb. 1, 2024; Published online Feb. 14, 2024

try their best to escape the infection of phages, while phages resist the immune defense of bacteria at full strength, or hunt for new hosts. This review focuses on the respective defense and anti-defense mechanisms of bacteria and their viruses. Their mutual competition and evolution eventually facilitate each other's adaptive evolution.

2 Mechanisms by which bacteria escape phage infection

Host bacteria can escape phage infection in many ways, which can be categorized by the time point of virus infection. The complete phage lytic cycle includes reversible and irreversible adsorption to the bacterial surface, the release of the phage genome, phage gene replication, and capsid biosynthesis, as well as the assembly and release of descendent phages.

2.1 Modification of cell surface structure

Most phages belong to the Caudovirales. All known long-tailed phages of Gram-negative bacteria first adsorb to a protein receptor molecule on the bacterial surface. Contractible-tailed phages of Gram-positive bacteria bind to a polysaccharide receptor, but some contractible-tailed phages of most Gram-negative bacteria also bind to polysaccharide receptors (Bertozzi Silva et al., 2016). All known short-tailed phages bind to polysaccharide receptors to initiate the downstream injection and release of the phage genome (Andres et al., 2010).

Hypermutable loci are widely present in bacteria. As one of the mechanisms of rapid phenotypic diversity in bacterial populations, these enable bacteria to survive under variable, usually antagonistic selection pressure (Turkington et al., 2019). Due to the high frequency of expression and the diversity of phage receptors on the surface of bacteria, local hypermutation sites can facilitate escape from phage adsorption.

2.1.1 Preventing phage binding by alteration of surface structures such as capsules

Capsular polysaccharides (CPSs) are key determinants of bacterial virulence and phage susceptibility (Lees-Miller et al., 2013; Geisinger and Isberg, 2015; Volozhantsev et al., 2020; Talyansky et al., 2021). It has been reported that *Campylobacter jejuni* 11168R, which escapes the infection of phage F336, can block the adsorption of F336 by modifying the phosphate on its surface. The treatment of 11168R with protease K was found to have no effect on the adsorption of F336, but its adsorption decreased after treatment with periodate which can degrade polysaccharides. These results show that the receptor of phage F336 is a polysaccharide rather than a protein (Sørensen et al., 2011). Another study with similar conclusions dealt with a clinical strain of *Acinetobacter baumannii* and its phages ΦFG02 and ΦC001, which bind to the capsule of the host bacteria. Bacterial variants can resist ΦFG02 and ΦC001 infection via the deletion of functional genes in the K locus, resulting in the impairment of CPS and deletion of the capsule, which finally affects the adsorption of phages ΦFG02 and ΦC001 (Gordillo Altamirano et al., 2021). Similarly, Wang et al. (2021) discovered that *A. baumannii* cannot be killed by the phage Phab24 after mutation of the *gtr9* gene located in the K locus, showing that bacteria can change their CPSs to resist phage infection.

2.1.2 Modifying lipopolysaccharides or outer membrane proteins on the surface to prevent phage adsorption

Lipopolysaccharide (LPS) includes lipid A, core antigen, and O-antigen, among which the O-antigen contains repeated polysaccharide polymers (Kenyon and Hall, 2013). A high level of O-antigen diversity was observed in *Escherichia coli*, which is the basis of serotyping and determines the receptor diversity of phages (Labrie et al., 2010). The clustered regularly interspersed short palindromic repeats (CRISPR) interference (CRISPRi) technology has enabled the screening and identification of *E. coli* genes required for phage infection, helping to identify known host bacteria factors and providing new insights into the interactions between phages and host bacteria. It was found that phage receptors and other genes involved in phage adsorption play the most effective role among the host genes involved in phage infection. However, this screening method has limitations: it cannot identify the host bacterial genes that play a role in the later stages of phage infection when the guide RNA and transposon have been degraded (Rousset et al., 2018). Another study showed that phage Phab24 of *A. baumannii* cannot infect *gtrOC3*-mutated variants, but phage adsorption was unaffected. Since *gtrOC3* is involved

in the synthesis of lipooligosaccharide (LOS) and serves as the secondary phage receptor, it is likely that bacteria can escape the lysis of phages by modifying LOS (Wang et al., 2021). A study on the phage resistance mechanism of *Klebsiella pneumoniae* revealed that a single nucleic acid base deletion of the gene encoding the outer membrane protein C (OmpC) resulted in resistance to phage GH-K3 (Cai et al., 2018).

Except for the phage receptors of *E. coli*, which have been studied in great detail, little is known about the phage receptors of bacteria, and the evolutionary path by which phages adapt to the mutation of their receptors remains to be revealed.

2.2 Preventing entry of the phage genome

The superinfection exclusion (Sie) system contains some proteins encoded mainly by prophages, with some encoded by lytic phages, such as the T4 phage (Dulbecco, 1952; Anderson and Eigner, 1971; Lu and Henning, 1994). These proteins are considered to anchor the bacterial cell membrane or membrane-related components and block the injection of the phage genome (van Houte et al., 2016). Another Sie example comes from *Salmonella typhimurium* and its short-tailed phage P22. The SieA is a membrane-associated protein that can block injection of the phage P22 DNA via interaction with the trans-membrane channel. Since the phage P22 cannot directly deliver the phage genome into the cytoplasm, the construction of the trans-membrane channel is an effective procedure, especially for Gram-negative bacteria which have a cell wall and two membranes (Swanson et al., 2021), while the host bacteria smartly harness the SieA to prevent the formation of the trans-membrane channel (Wang et al., 2019). The SieA belongs to a group of P22-borne superinfection exclusion factors (SEFs). Staes et al. (2022) revealed that P22 can take advantage of carrier-state dynamics and its expression of the Sie to enhance subpopulations of host cells for lytic consumption (Staes et al., 2022). Nonetheless, the Sie system also can assist in infection by other kinds of phages (Labrie et al., 2010).

2.3 Cutting the phage genome or preventing phage DNA replication

2.3.1 Restriction-modification system

The restriction-modification (RM) system exists in most bacteria. Its core components are enzymes that can specifically recognize and cut exogenetic DNA while simultaneously modifying and protecting bacterial DNA. Some RM enzymes have been synthesized and applied in the biomedical field. The RM system can be divided into four types (I to IV). It is a diversified and extensive innate immune system existing in bacteria, which "eliminates dissidents" by cutting non-self and non-modified DNA (Labrie et al., 2010). Most RM systems consist of two components: the first is the enzyme or methyltransferase (MT) that methylates DNA. DNA methylation is the process of adding methyl (CH_3) groups to DNA molecules. The sequence of DNA fragments does not change, but DNA methylation can change the role of the DNA. The second component is a restriction endonuclease (RE), which can recognize specific DNA sequences or restriction sites and specifically cut unmethylated DNA (Nasrullah et al., 2022). To protect the bacterial genome from being digested by RM enzymes, most RM enzymes also have DNA MT activity, which methylates the same sequence as the target of the endonuclease. Therefore, the role of MT is to block the endonuclease from cutting the bacterial DNA, but it can freely attack exogenous unmethylated viral DNA (van Houte et al., 2016). In a classical RM system, methylation usually occurs in a palindrome sequence, which means that both DNA strands are modified on the same sequence. The methylase can recognize the hemimethylation site during bacterial DNA replication, so that the newly synthesized unmethylated strand paired with the methylation site on the old strand will also be methylated (Papoulis et al., 2021). However, the Rotem SOREK team found a non-classical RM system based on methylation of non-palindromic sequences in *Bacillus cereus* (Goldfarb et al., 2015). This bacteriophage exclusion (BREX) system contains a core element composed of six genes: an encoding Lon-like protease, RNA-binding protein, alkaline phosphatase domain protein, DNA methylase, ATPase domain protein, and a protein with unknown function. The phage DNA was only blocked by the BREX system, not cut or degraded. They also found that a family of phages can escape the BREX system (Goldfarb et al., 2015).

2.3.2 CRISPR/Cas system

CRISPR sites are derived from sequences of foreign invading phages or plasmids and separated by common repeats, and can provide a genetic memory of previous infection. CRISPR-associated (Cas) genes

encode proteins that perform an immune response (van Houte et al., 2016). At least 50% of bacteria and the most archaea have this acquired immune system (Koonin and Krupovic, 2020).

Cas10 from the *Staphylococcus* III-A CRISPR/Cas system can promote the genomic mutation of *Staphylococcus* and accelerate its resistance to antibiotics. Cas10 is able to cut the genome of invaders in a non-specific manner which results in random single-stranded DNA (ssDNA) fragmentation, which finally allows the evolution of *Staphylococcus* (Mo et al., 2021). In addition, researchers proposed that the type III-A CRISPR/Cas system of *Staphylococcus* not only confers phage resistance, but also has additional functions (Mo et al., 2021). They speculated that type V Cas12 and Cas14 can also evolve this nonspecific cleavage activity of Cas10, so as to improve the mutation rate of host bacteria and promote adaptation to a changing external environment and threats from new phages.

The CRISPR/Cas system protects bacteria from phage invasion, but some scholars have proposed that it cannot distinguish between self and non-self DNA in the course of acquiring spacers, and occasionally obtains the spacer sequence of the bacterium itself. Moreover, the CRISPR/Cas system inhibits bacteria from acquiring foreign DNA, which often mediates pathogenicity and antibiotic resistance (Li and Bondy-Denomy, 2021). Apart from that, CRISPR/Cas immunity has been proved to be unexpectedly beneficial to phages by increasing the mutation rate of phages to accelerate phage evolution. The CRISPR/Cas system may hinder the acquisition of beneficial genes by bacteria and may also lead to a risk of autoimmunity (Bondy-Denomy et al., 2013; Seed et al., 2013).

Since CRISPR/Cas was discovered, its application has been excavated continuously, which has greatly enriched the toolbox of gene editing (Gao et al., 2022). It is reported that the CRISPR/Cas system has been combined with the lambda red recombination system and applied to small fragment single nucleotide mutation. The three-plasmid method developed by researchers not only can effectively recombine short single-stranded oligonucleotides, but also can use large polymerase chain reaction (PCR) products to recombine and substitute polygenic chromosome fragments, such as a 19.4-kb DNA deletion containing 19 non-essential chromosome genes and a 3-kb heterologous DNA insertion (Pyne et al., 2015). The recombination efficiency can be verified by colony PCR. This three-plasmid approach can produce markerless and scarless gene replacement mutants. On the other hand, phage genomes can also be engineered based on phage-derived recombinant proteins and CRISPR/Cas9 of *Streptococcus thermophilus*. Wetzel et al. (2021) described a phage genome recombination method based on the electroporation DNA CRISPY-bacteriophage recombining of electroporated DNA (BRED) and phage gene recombination method, based on the infection particle CRISPY-bacteriophage recombining with infectious particles (BRIP), to effectively and accurately transform *Mycobacterium* phage, and this method is also applicable to the modification of phages of other bacteria (Wetzel et al., 2021).

de Freitas Almeida et al. (2022) co-evolved the bacterial fish pathogen *Flavobacterium column* and its lytic phage V156 for up to 16 weeks in the presence and absence of a eukaryotic host signal (mucin). They found that the presence of mucin resulted in a dramatic increase in CRISPR spacer acquisition, especially under low-nutrient conditions, with more than 60% of colonies acquiring at least one new spacer. The presence of competing bacteria further increased the acquisition of CRISPR spacers in *F. column*. Their study indicated that while surface modification is the primary phage resistance mechanism in bacteria, the mucosal environment and bacterial competition significantly increase CRISPR spacer uptake.

2.3.3 Prevention of phage DNA replication

Unlike the defense systems listed above, which rely on protein components, Kronheim et al. (2018) found a natural molecule or secondary metabolite (doxorubicin, actinomycin D, cosmomycin D) widely existing in *Streptomyces*, which can target all double-stranded DNA (dsDNA) phages by inserting into DNA to prevent replication. Since these secondary metabolites are diffusible molecules produced by most bacterial species, they can provide an innate defense system to protect the entire bacterial community (Kronheim et al., 2018). Tal et al. (2022) discovered a family of defensive bacterial deoxycytidine triphosphate (dCTP) deaminase proteins that convert dCTP to deoxyuracil nucleotides upon phage infection. They also discovered a family of phage resistance genes encoding deoxyguanosine triphosphatase (dGTPase), which degrades

deoxyguanosine triphosphate (dGTP) to phosphate-free deoxyguanosine (Tal et al., 2022). They propose that, during phage infection, the bacterium's defense proteins deplete specific deoxynucleotides (dCTP or dGTP) from the nucleotide pool, starving the phage of the necessary DNA-building blocks, thereby stopping its replication.

2.4 Preventing the release of mature phage particles by earlier self-lysis

A good example of "self-sacrifice" is the abortive infection (Abi) system. Through the Abi system, infected bacteria will lyse to deter phage reproduction, thus "sacrificing" a few bacteria in exchange for the survival of the entire bacterial population (Labrie et al., 2010). The Abi system involving *E. coli* and lambda phage has been fully studied and is named the Rex system. The silenced RexA protein can be activated by the DNA protein complex of exogenous phage. The membrane protein RexB, which plays the role of ion channel, is then activated and the cation causes the depolarization of the cell membrane and final cell death by activation of RexB (van Houte et al., 2016).

Additionally, bacteria regulate their own death process by generating cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) (cGAMP) signal. The system is composed of a four-gene operon encoding cGAMP synthase and related phospholipases, as well as two enzymes with eukaryotic domains E1, E2 and Jun activation domain-binding protein (JAB) (Cohen et al., 2019). The operon endows bacteria with tolerance to phages. Phage infection leads to cGAMP biosynthesis, and cGAMP activates phospholipase, resulting in the destruction of the cell membrane and bacterial death before phage replication. The mechanism by which the bacterial immune system detects phage components has not been clarified, but it is certain that the phage component that can be detected is unlikely to be phage dsDNA, since the bacterial cytoplasm contains its own dsDNA.

Another recent discovery is that bacteria resist phages and execute cell death through pyroptosis. Gasdermin protein (GSDM) forms large holes in human cell membranes, releases immune cytokines, and induces cell death. Johnson et al. (2022) reported that once the GSDM homologue encoded by bacteria is activated, it can also lead to membrane rupture and bacterial pyroptosis. Therefore, they believed that pyroptosis is an ancient and regulated form of cell death shared between bacteria and animals (Johnson et al., 2022).

Abi also can be embodied in the Thoeris defense system. This system is widely distributed in bacteria and contains two essential genes, *thsA* and *thsB*. *thsA* has a nicotinamide adenine dinucleotide (NAD)-binding domain, and *thsB* has the Toll/interleukin-1 receptor (TIR) domain (Doron et al., 2018). de Freitas Almeida et al. (2022) have proposed that Thoeris is the prokaryotic ancestral form of pathogen-associated molecular pattern (PAMP) receptors. This potential anti-phage mechanism has been deciphered by the Rotem Sorek group. They discovered that phage infection triggered the Thoeris TIR domain to biosynthesise the cyclin adenosine diphosphate (ADP)-ribose (ADPR) isomer, and then activate the second molecular ThsA, followed by clearing the critical NAD, finally leading to bacterium death and Abi (Ka et al., 2020; Ofir et al., 2021).

2.5 Promoting phage adsorption by producing extracellular "bait"

Some bacteria can produce vesicles and squeeze them out of the cell. The membrane of these vesicles has phage receptors that promote phage adsorption, thereby greatly reducing the chances of phage adsorption to the bacterial surface (Nguyen et al., 2017). Furthermore, some bacteria use the prophage to encode small molecule secretory peptides, which will combine and react with the offspring phages, resulting in the offspring phages no longer infecting the original host bacteria (Le et al., 2020).

Some scholars have used mathematical models to show that the existence of bacterial fragments in the phage-bacteria system can provide a safe haven for the coexistence of bacteria and phages. The "suicide" infiltration of phages adsorbed to bacterial fragments will not completely eliminate phages or bacteria. The researchers also believe that there is a group of initial unmodified phages. While bacteria sensitive to initial phages still exist, they are found only on a small scale (Rabinovitch et al., 2003).

2.6 Toxin and antitoxin systems

Toxin and antitoxin (TA) systems are small modules composed of a stable toxin and an unstable

antitoxin. The toxins act as messenger RNA (mRNA) interferases that impact protein biosynthesis, while the antitoxins can counteract the toxins (Unterholzner et al., 2013). TA systems were originally described on plasmids, and then chromosomal TA modules were discovered that can protect against invading phages and plasmids (LeRoux and Laub, 2022). At present, based on the mechanism of how antitoxins neutralize toxin activity, TA systems are divided into six categories (I to VI). Although the products of toxin genes are usually proteins, the products of antitoxin genes are mainly non-coding RNAs (types I and III) and low-molecular-weight proteins (types II, IV, V, and VI) (Page and Peti, 2016). The gene locus of the TA module plays a key role in the persistence of bacteria. Under normal growth conditions, an antitoxin can effectively inhibit the activity of a toxin. In contrast, under stress conditions, antitoxins are selectively degraded and released to inhibit necessary cellular physiological processes, such as DNA replication and translation. The resulting non-dividing cells can survive environmental stress, such as nutrient deficiency or phage exposure. Once the pressure is relieved, the persistent bacteria return to an active growth state and reproduce the original population.

2.7 Phage-induced chromosome islands

Aiming to prevent virus-mediated host takeover, bacteria have developed a phage defense system comprising mobile genetic elements (MGEs), which often gather on the mobile defense island of the bacterial genome (McKitterick et al., 2019). PICIs have been deeply studied in *Staphylococcus aureus*, also known as *S. aureus* pathogenic islands (SaPIs). Phage-induced chromosome islands (PICIs) are integrated into the bacterial chromosome and excised with the help of a specific "helper phage" induced by infection or lysogen. PICIs interfere with the life cycle of the phage and so can be regarded as a mechanism to resist phage infection (Rostøl and Marraffini, 2019). PICIs are usually small and only encode factors that can be excised and integrated into the bacterial chromosome. These factors can promote the packaging and transmission of PICIs and inhibit the expression of their own genes without auxiliary phages (Rostøl and Marraffini, 2019).

The viral satellites of *Vibrio cholerae*, also known as PICI-like elements (PLEs), destroy its cleavage phage ICP1, triggering the PLEs to be removed from the bacterial genome, and then reduce the production of phages by replication and transduction to adjacent bacteria, ultimately protecting the host bacteria (McKitterick et al., 2019).

The above seven bacterial defense mechanisms against phage infection have been summarized (Table 1 and Fig. 1) and can be divided into two categories: constitutive defense mechanisms (e.g., CRISPR/Cas) and inducible defense mechanisms (e.g., modification of bacterial surface receptors). By applying theoretical models and experimental evolution, Westra et al. (2015) proposed a mechanism to drive the evolution of phage defense, indicating that the risk of phage infection determines the relative investment of the two

Table 1 Summary of the defense mechanisms of bacteria

Mechanism	Representative cases and references
Surface modification	CPS (Sørensen et al., 2011; Gordillo Altamirano et al., 2021; Wang et al., 2021); LPS (Labrie et al., 2010; Wang et al., 2021); OMP (Cai et al., 2018)
Preventing phage genome injection	Sie system (Dulbecco, 1952; Anderson and Eigner, 1971; Lu and Henning, 1994; Wang et al., 2019)
Preventing phage genome replication	RM (Goldfarb et al., 2015; Nasrullah et al., 2022); CRISPR/Cas (Mo et al., 2021; de Freitas Almeida et al., 2022); Host nucleotide depletion (Kronheim et al., 2018; McKitterick et al., 2019; Rostøl and Marraffini, 2019; Tal et al., 2022)
Preventing mature phage particle release	TA system (Page and Peti, 2016; LeRoux and Laub, 2022); Abi system (van Houte et al., 2016; Doron et al., 2018; Cohen et al., 2019); GSDM (Johnson et al., 2022)
Baiting phage binding by secretion of vesicles	Rabinovitch et al., 2003; Nguyen et al., 2017; Le et al., 2020

CPS: capsular polysaccharide; LPS: lipopolysaccharide; OMP: outer membrane protein; Sie: superinfection exclusion; RM: restriction-modification; CRISPR: clustered regularly interspersed short palindromic repeats; Cas: CRISPR-associated proteins; TA: toxin and antitoxin; Abi: abortive infection; GSDM: gasdermin protein.

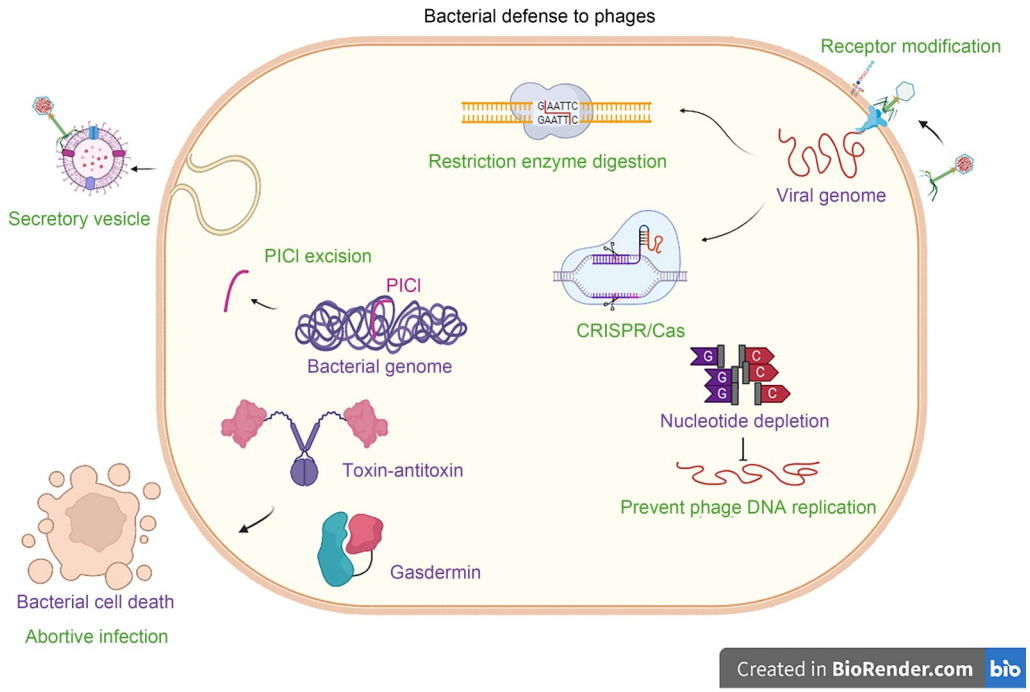

Fig. 1 Schematic of bacterial defense to phages. PICI: phage-inducible chromosome island; CRISPR: clustered regularly interspersed short palindromic repeats; Cas: CRISPR-associated proteins.

defense methods. Under high-frequency phage exposure, the CRISPR/Cas system, as a constitutive defense type, has obvious limitations. For example, it provides only partial immunity, which is easier to overcome by phage evolution and has more adaptive costs than inducible immunity. Westra et al. (2015) also believed that the extent of exposure to parasitic organisms may be the key factor to promote the evolution of constitutive or inducible defense in nature. Organisms living in an environment rich in parasitic species are more likely to evolve constitutive defense mechanisms, whereas when parasites are lacking, they are prone to evolve inducible defense mechanisms (Westra et al., 2015).

3 Mechanisms of phage resistance to bacterial immunity

Although bacteria can produce immunity or resistance to phages, phages can also evolve anti-defense mechanisms against bacteria.

3.1 Changing the receptor-binding proteins of the phage

Bacteriophages typically have multiple types of receptor-binding proteins (RBPs) that form tail fibers or tail spikes that are connected to the baseplate through the N-terminal domain. The C-terminal domain is exposed to the environment and participates in the recognition and binding of bacterial surface receptors (de Jonge et al., 2019; Dunne et al., 2019). Unlike the tail fibers, the tail spikes have enzymes, which not only bind to the protective polysaccharide on the bacterial surface, but also degrade it. The degradation of CPS exposes the secondary phage receptor on the bacterial surface, which is recognized by other proteins of the phage and completes irreversible adsorption (Wang et al., 2021). Phages of the former family Podoviridae often have depolymerases as part of their structure. These depolymerizing enzymes appear in the form of tail spike proteins (TSPs) (Knirel et al., 2020). After specific binding with the CPS, extracellular polysaccharide (EPS), or LPS of the host bacteria, the repeated unit of the polysaccharide is specifically cut, and then the phage threads the last barrier—the cell wall, and finally injects the its DNA and hijacks the anabolic machinery of the host (Majkowska-Skrobek et al., 2016).

The RBPs of the phage can be specifically matched with bacterial surface receptors, like keys and locks. A single RBP can bind to multiple receptors, and a receptor can be recognized by multiple RBPs. If the

receptor is lost or modified, it cannot bind to the corresponding RBP, and there will be no phage infection, providing a way for bacteria to resist phages. However, phages can mutate their related genes and produce novel RBPs that bind to new receptors.

Based on the tail protein composition, the host range of phages can be redesigned (Xie et al., 2018). Some phages change the specificity of binding to receptors by altering their RBPs, so the modification of RBPs by genetic engineering provides an important means to change the host range of phages. The C-terminal receptor-binding domain of an RBP is generally changed by homologous recombination. Ando et al. (2015) applied a synthetic biology strategy, using an efficient and simple yeast-based phage engineering platform, to adjust the host range of several members of the T7 phage family. They reconstructed phages that can target pathogenic *Yersinia* and *Klebsiella* bacteria using the scaffold of *E. coli* phage, and used the scaffold of *Klebsiella* phage RBP at the same time combined with the tail fiber component of *E. coli* phage to cleave *E. coli*. In classical phage hybridization or recombination experiments, screening ideal phage mutations may require hundreds of individual plaques for PCR, restriction enzyme digestion, or plaque hybridization, which is time-consuming and laborious. The method of Ando et al. (2015) rarely requires screening many yeast clones, because their experimental data show that at least one quarter of yeast clones contain correctly assembled phage genomes (composed of up to 11 DNA fragments), which can be activated into functional phages after transformation into bacteria. This synthetic phage realizes the effective infection of new target bacteria, but the authors also mentioned the limitations of this strategy, such as the need to reboot the modified phage genome into a functional phage, and the need to identify loci that can be manipulated to change the host range of phages (Ando et al., 2015). It is difficult to alter the host range of long-tailed phages (Leitner et al., 2021). Their RBP is an integral part of the baseplate and undertakes the functions of phage adsorption and DNA transport. Because the tail spike participates in various protein–protein interactions in the baseplate, the modification of the tail spike would affect the integrity of macromolecular complexes and hinder the formation of infectious virus particles.

3.2 Forming a physical barrier to avoid the degradation of bacteriophage DNA by bacteria

Phages can establish corresponding physical barriers to avoid damage from host bacteria. The area protected by physical barriers is the specific area where the phage genome can replicate safely. Mendoza et al. (2020) found that *Pseudomonas aeruginosa* giant phage ΦKZ can assemble a protein barrier around its genome to escape the targeted DNA enzymes of bacteria. This barrier can separate the restriction endonuclease of host bacteria from the Cas protein and phage genome throughout the infection process. The authors also mentioned that the DNA of the phage will never be directly exposed to cytoplasm. However, note that the type VI CRISPR/Cas system can still target phage mRNA in the presence of the protective barrier.

Malone et al. (2020) found a giant phage of *Serratia* that can escape the type I CRISPR/Cas system but is sensitive to the type III system. This giant phage can also form a nucleus-like barrier structure, which helps it escape the targeting of the type I CRISPR/Cas system. However, this nucleus-like structure is sensitive to the type III CRISPR/Cas system that can target RNA. The phage "nucleus" is an anti-defense strategy widely used by giant phages. This mechanism of immune escape has led people to predict that phage nuclei could provide extensive protection from various immune systems targeting DNA. Therefore, phages that can form nucleus-like structures could be considered promising candidates for phage therapy.

3.3 Anti-CRISPR systems

3.3.1 Phages can escape the CRISPR/Cas system by mutating related sequences

Phages can mutate related sequences, thus losing complementarity with bacterial genome spacer sequences, resulting in failure to be recognized by the bacterial CRISPR/Cas system (Cho, 2014). The CRISPR locus is composed of short DNA repeats, namely spacer sequences, which are matched with the genome of a phage or foreign plasmid. The spacer sequences are transcribed and processed to generate guide RNA. CRISPR-related nucleases recognize and target the externally invading complementary nucleic acid fragments (Yang and Patel, 2017). A template phage ΦAP1.1

prevents Cas9 function by expressing a small anti-CRISPR protein AcrIIA23, allowing it to integrate into the direct repeat unit of the CRISPR locus to neutralize the CRISPR-Cas9 immunity of bacteria. A bioinformatic method showed that ΦAP1.1 is integrated not only into CRISPR repeats, but also into Cas genes to form prophages. This destruction of CRISPR/Cas sites through prophages may be a common mechanism of anti-bacterial immunity (Varble et al., 2021). On the other hand, as phages have a rapid proliferation rate and a large population size, it is easy to accumulate mutations in homologous target sequences (e.g., precursor sequence, protospacer) to avoid CRISPR/Cas targeting, but this approach incurs a greater fitness cost. Moreover, to meet the requirements of coexistence with diverse bacteria in the natural environment, it seems unlikely that phages will mutate many sites (Li and Bondy-Denomy, 2021). Fineran et al. (2014) discovered that in *E. coli* K12, the intruder can make a point mutation in the leading sequence to avoid type I-E CRISPR/Cas immunity, but host bacteria can quickly restore immunity by integrating a new spacer sequence via a positive feedback process called "priming." Priming is extremely powerful, even if it contains many mismatched spacer sequences, as it can also induce CRISPR to quickly resist a variety of related phages, giving bacteria advantages in the arms race with their viruses (Fineran et al., 2014). Therefore, some scholars have suggested that phages need a more complex mechanism to resist CRISPR (Li and Bondy-Denomy, 2021).

3.3.2 Phages can also inhibit CRISPR/Cas activity

To escape the CRISPR/Cas system of bacteria, bacteriophages overcome the adaptive immunity of bacteria by encoding an anti-CRISPR (Acr) protein, inhibiting the assembly of the CRISPR/Cas complex, blocking target binding, and preventing target cleavage (Hirschi et al., 2020; Jia and Patel, 2021). Acr protein inhibits CRISPR/Cas activity through a variety of mechanisms, so that phages can successfully replicate through the replicative lytic or lysogenic cycles. Acr protein is a typical small protein, generally containing 80–150 amino acids, which binds directly and then inactivates the target Cas protein (Li and Bondy-Denomy, 2021).

Meeske et al. (2020) reported that AcrVIA1, an anti-CRISPR protein encoded by *Listeria* phage (LS46), can inactivate the Listeria type VI-A CRISPR system. AcrVIA1 prevented the binding of targeted RNA and subsequent conformational changes of Cas13a after binding with Cas13a. Unlike the limited immunosuppression mediated by DNA-cleaved Cas nuclease inhibitors and the need for multiple infections to resist CRISPR defense, the anti-CRISPR effect mediated by the Cas13a inhibitor AcrVIA1 is more powerful and requires less AcrVIA1 (Meeske et al., 2020).

3.3.3 Phages can neutralize the CRISPR system by degradation of cyclic oligonucleotide signal molecules

Athukoralage et al. (2020) pointed out that a new enzyme, AcrIII-1, can rapidly degrade cyclic tetraadenylate (cA4) and is widely distributed in archaea and bacterial viruses. The enzyme uses a previously unknown folding mode to specifically bind to the signal molecule cA4. The conserved active site can quickly cut the cA4 so that the phage can neutralize the type III CRISPR defense system.

3.4 Resisting the RM system by preventing DNA methylation of host bacteria

S-Adenosylmethionine (SAM) lyase (SAMase) of phage T3 degrades the SAM pool in host *E. coli* cells, thereby inactivating key metabolites involved in many cell functions, including DNA methylation. The main biological role of SAMase is to make T3 phages immune to host bacteria (Studier and Movva, 1976). Single particle cryo-electron microscopy and biochemical experiments have shown that SAMase not only degrades SAM, but also exerts its function by interacting with methionine *S*-adenosyltransferase (MAT) of the host bacteria and effectively inhibiting the enzyme producing SAM (Simon-Baram et al., 2021). Note that phages lacking SAMase can still overcome the RM system of host bacteria as effectively as phages expressing the enzyme (Krüger and Schroeder, 1981), but the specific mechanism remains to be clarified.

3.5 Degradation of the chromosome of host bacteria

The direct degradation of the host chromosome by bacterial viruses is a unique and irreversible host takeover process. It not only digests and releases nucleotides that can be incorporated into the rapidly replicating phage genome, but also destroys the template required for the expression of anti-phage genes encoded by host bacteria (McKitterick et al., 2019), effectively

"killing two birds with one stone." Bacterial viruses can directly degrade the chromosome of host bacteria and use the degraded nucleotides to synthesize phage DNA. This degradation is irreversible. In contrast, eukaryotic viruses degrade only the transcriptional RNA and have no effect on the DNA of host cells (Yang et al., 2021).

3.6 Son of Sevenless (SOS)-mediated prophage induction

When host bacteria are faced with a harsh environment, the prophage in the genome of the host bacteria will be induced into a lytic phage and released from the "dying" host bacteria. This is one of the strategies of the prophage to protect itself from extinction (Rocchi et al., 2019; Chevallereau et al., 2022). Nevertheless, the understanding of how prophages perceive adverse factors is very limited (Wahida et al., 2021). Many researchers claim that mitomycin C or ultraviolet (UV) irradiation is used to induce the prophages in bacterial chromosomes (Turkington et al., 2019; Chevallereau et al., 2022). Some host bacteria contain multiple prophages (Loh et al., 2020). Can all prophages be induced, or can one or some prophages be induced under some specific selection pressure? How can the induced prophage find a new host when the original host is almost destroyed? Is this strategy of being forced to abandon the original host beneficial only to phages or is it good for both parties? These questions need more research and exploration.

3.7 Inhibiting TIR-gcADPR signaling

To resolve Abi, the phage can produce proteins to block the bacterial immune signal transduction. These proteins, from the protein family named Thoeris anti-defense 1 (Tad1), can bind and sequester the signaling molecule produced by TIR-domain proteins and thereby evade the bacterial Thoeris defense system. Leavitt et al. (2022) solved the Tad1 structure and discovered that Tad1 can bind to the glycocyclic ADPR (gcADPR).

The above mechanisms of phage resistance to bacterial immunity have been integrated in Table 2 and Fig. 2.

4 Cooperation between bacteriophages and bacteria

Although bacteria and phages have defense and anti-defense interaction modes, respectively, they continue to co-evolve. As in the classic example of the Red Queen hypothesis, predators and prey must continue to evolve (Rohwer and Segall, 2015). Bacteriophages exert continuous pressure on bacteria, and then bacteria fight back through various defense methods. Phages evolve new selection pressure to which bacteria respond, and then they finally adopt cooperation and maintain a balance in the process of evolution (Maxwell, 2019). Similarly, some scholars have proposed that the relationship between phages and their bacterial hosts in the gut is best characterized not by death struggles between enemies, but by reciprocity between partners (Shkoporov et al., 2022). They suggested that despite the apparent selfish and exploitative nature of individual phages towards individual bacteria, phages and bacteria have co-evolved at the population level and thus benefit from their coexistence. Phage selection pressure leading to bacterial genotypic and phenotypic diversification, the use of phage lysis as a weapon against competitors or a

Table 2 Summary of the anti-defense mechanisms of phages

Mechanism	References
RBP alteration	Ando et al., 2015; Knirel et al., 2020
Protecting phage genome by physical barrier	Mendoza et al., 2020
Anti-CRISPR	Fineran et al., 2014; Athukoralage et al., 2020; Hirschi et al., 2020; Meeske et al., 2020; Jia and Patel, 2021; Varble et al., 2021
Preventing host DNA methylation	Studier and Movva, 1976; Simon-Baram et al., 2021
Degrading host chromosome	Yang et al., 2021
Prophage induction	Rocchi et al., 2019; Chevallereau et al., 2022
Inhibiting the TIR-gcADPR signaling	Leavitt et al., 2022

RBP: receptor-binding protein; CRISPR: clustered regularly interspersed short palindromic repeats; TIR: Toll/interleukin-1 receptor; gcADPR: glycocyclic adenosine diphosphate ribose.

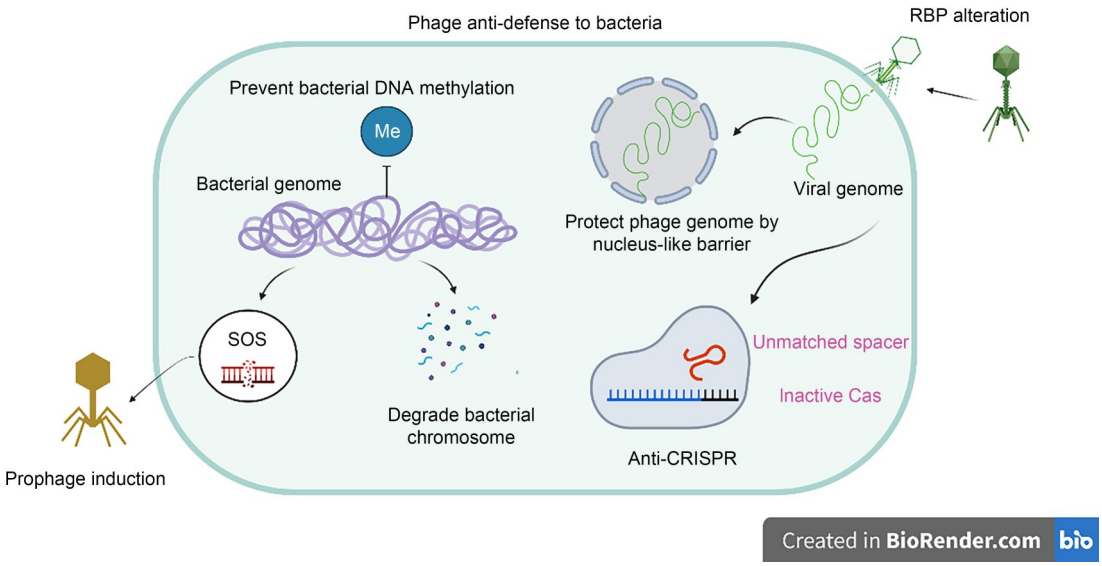

Fig. 2 Schematic of phage anti-defense to bacteria. RBP: receptor-binding protein; CRISPR: clustered regularly interspersed short palindromic repeats; Cas: CRISPR-associated proteins; SOS: Son of Sevenless.

means of interacting with metazoan hosts, and the acquisition of a broader pan-genome through phage transduction, all provide bacterial populations with unique benefits.

We should think critically about the relationship between bacteriophages and bacteria. There is no eternal enemy in nature. The proteins encoded by the genes carried by phages can regulate some physiological functions of host bacteria, such as metabolism and antibiotic resistance (Labrie et al., 2010). Meanwhile, the host bacteria can also provide places and material for the survival of phages (Bodner et al., 2021). Sweere et al. (2019) revealed that the filamentous phage Pf of *P. aeruginosa* promotes host bacterial infection by directly affecting mammalian immunity. The Pf phage inhibits the effect of tumor necrosis factor (TNF) in a type I interferon-dependent manner. They also reported that the direct interaction between internalized phages and immune cells may occur in a way that seriously affects human health, assuming that human cells may contain internalized phages produced by symbiotic and pathogenic bacteria. This study showed the importance of internalized phages in bacterial infection and suggested that phages may have a far-reaching and direct impact on human health. Another study on the mutual assistance between phages and bacteria also comes from phage Pf and its host bacteria. Secor et al. (2015) showed that Pf can promote the assembly and function of *P. aeruginosa* biofilms. They showed that the extracellular matrix produced by *P. aeruginosa* is self-assembled into a crystal-like structure through the interaction between polymer and negatively charged filamentous phage Pf. This structure can increase the host bacteria's tolerance to desiccation and antibiotics, thereby enhancing the function of the biofilm and the adaptability of the host bacteria. Pf is widespread in clinical isolates of *P. aeruginosa* and detected in the sputum of patients with cystic fibrosis (CF), so researchers believe that targeting filamentous phage or biofilm crystal-like tissue is a potential antibacterial strategy (Secor et al., 2015). In addition to *P. aeruginosa*, other Gram-negative bacteria such as *E. coli* and *V. cholerae* can produce filamentous phages, which may result in other chronic infections by affecting the structure and function of biofilms (Tian et al., 2015; Bisht et al., 2021). This is a win-win evolutionary pattern: *P. aeruginosa* can survive in an unfavourable environment, and Pf phage can persist in the host bacteria. However, other researchers deem that the increase in biofilm formation of *S. aureus* and *S. typhimurium* seems to be the result of non-evolutionary mechanisms. The impact of bacteriophages on biofilms depends on the environment and is jointly controlled by evolutionary and non-evolutionary mechanisms (Hosseinidoust et al., 2013). Low level exposure to phages can produce a biofilm-enhanced environment and protect *S. aureus* from complete elimination. The result will be

a balance, which will not only help the bacteria resist adverse environmental factors, but also retain the bacterial library which is sensitive to phages. When dormant bacteria are reactivated, phages can also capture sensitive host bacteria (Fernández et al., 2017).

Dragoš et al. (2021) conducted high-throughput biosynthetic gene cluster (BGC) mining on all sequenced phage and prophage genomes and, combined with experiments, proved that most prophage BGCs encode bacteriocins to help bacteria survive better, while phages use BGCs to arm host bacteria through lysogenesis. Owen et al. (2021) identified a phage defense protein family BstA encoded by prophage in various Gram-negative bacteria. BstA is located at the site of exogenous phage DNA replication, mediates Abi, and restrains the prevalence of competitive phages. In the process of cleavage replication, the prophage encoded by BstA itself is not inhibited by BstA, which is due to the self-immunity given by anti-BstA (aba), a small piece of DNA in the BstA site. The inhibitory effects of different BstA proteins from *Salmonella*, *Klebsiella*, and *E. coli* prophages on phage replication are usually interchangeable, but each protein has homologous aba elements. This protein protects the host bacterial population from attack by foreign competitive phages by mediating Abi but does not sacrifice the replicative and lytic ability of the prophage.

Apart from the classical lysis and lysogen cycles, the phage has another state, the "phage carrying state" or pseudolysogeny. That is, after the phage injects its genome, the phage does not participate in the lytic and lysogen cycle, but remains in the host cell as a non-replicative extrachromosomal element. This state may be a way to protect phages from damage from UV and high temperatures (Chevallereau et al., 2022).

In addition to shaping the composition of bacterial populations, bacteriophages are an important driving factor for bacterial evolution, not only because of the selective pressure they exert, but also by means of lysogen transformation, transduction, and destruction of host genes after they are integrated into the bacterial genome to become prophages (Carroll-Portillo and Lin, 2019; Hussain et al., 2021). The diversity and population density of bacteria and other phages competing for the same host resources will also affect the evolution of bacterial viruses (Chevallereau et al., 2022). Sant et al. (2021) co-cultured phages ΦJB01 with the host and other bacteria of different genotypes. They found that the phage could evolve the ability to infect any new host, but increasing the number of host bacteria slowed down the evolution of the phage. Phages co-cultured with a single non-sensitive strain could not evolve the ability to infect the bacteria, but in the case of two or three strains including the original host bacteria, the evolved phages could infect these strains. The existence of new potential hosts will exert selective pressure on the phage population with two potential outcomes: the phage population evolves into a "generalist" and can target multiple hosts, or it develops into a "specialist" with multiple subgroups, each targeting a specific host. Nonetheless, the selective mechanism of the two results is unknown.

5 Summary

In the long process of evolution, bacteria and phages have developed defensive and anti-defensive survival modes, respectively. Since bacteriophages were first discovered and described more than 100 years ago, the mechanisms of interaction between bacteria and bacteriophage have been continuously explored. Bacteria escape the infection of bacteriophages by modifying surface receptors and cutting foreign genes. At the same time, phages resist bacterial defense by changing RBPs and degrading host bacterial chromosomes. These studies have laid the foundation of molecular biology and enriched methods of genetic engineering. Research on phages is helpful to understand host bacteria and diseases. Bacteria provide a haven for the maintenance of bacteriophages which carry genes encoding proteins and regulate the basic physiological functions of the host, such as metabolism and antibiotic resistance. The mutual evolution model of bacteria and their viruses is worthy of further in-depth exploration.

Acknowledgments

This work was supported by the Western Medicine Program of the Zhejiang Provincial Health Commission (No. 2024KY592) and the Fundamental Research Funds for Central Universities of the Central South University (No. 2-2050205-19-361), China.

Author contributions

Xiaoqing WANG wrote the original draft and draw the figures. Sebastian LEPTIHN conceived and supervised the review. Both authors have read and approved the final manuscript.

Compliance with ethics guidelines

Xiaoqing WANG and Sebastian LEPTIHN declare that they have no conflict of interest.

This review does not contain any studies with human or animal subjects performed by either of the authors.

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