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REVIEW

Small nucleotide molecule-mediated interactions between bacteriophages and their hosts: mechanisms and implications

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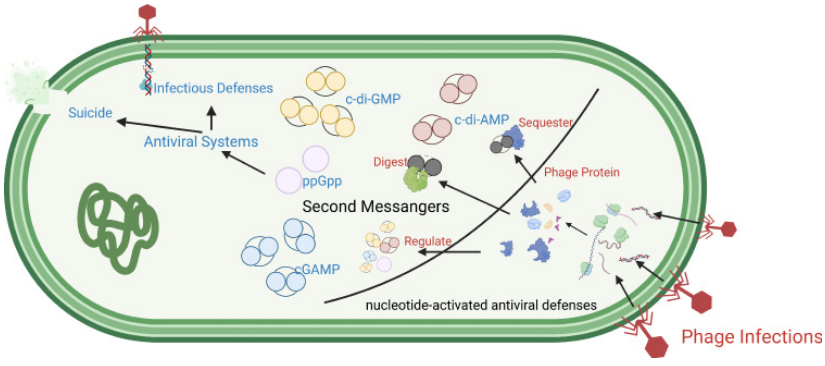
ABSTRACT: The perpetual molecular arms race between bacteriophages and their prokaryotic hosts revolves around nucleotide-derived signaling molecules that serve as pivotal regulators of offensive and defensive strategies. This review integrates contemporary advances elucidating how cyclic nucleotide monophosphates (cNMPs), bacterial second messengers (c-di-GMP, c-di-AMP, (p)ppGpp), and nucleotide-activated antiviral defense systems (CBASS, Thoeris, Pycsar, Kongming) coordinate phage-bacteria interactions through sophisticated molecular surveillance networks. We systematically analyze phage counteradaptation mechanisms that hijack nucleotide-mediated signaling pathways for replicative advantage and immune suppression, contrasted with bacterial counterstrategies employing nucleotide dynamics in restriction mechanisms and abortive infection systems. The review further examines cutting-edge biotechnological applications capitalizing on these molecular interactions, including precision phage therapeutics, engineered phage platforms, functional phage biodesign, and phage-mediated microbiome modulation. By synthesizing structural insights into nucleotide-based defense architectures with emerging phage resistance paradigms, we identify critical knowledge gaps regarding signal transduction specificity, evolutionary trade-offs in defense systems, and spatiotemporal regulation of nucleotide networks during lytic/lysogenic cycles. This comprehensive analysis provides a conceptual framework for advancing phage engineering and antimicrobial development in the post-antibiotic era.



KEYWORDS: cyclic nucleotide, phage defense, phage therapeutics, phage-bacteria interactions

1 Introduction

Bacteriophages (phages) and their bacterial hosts are locked in an evolutionary arms race fundamentally mediated by nucleotide signaling molecules. Crucially, many of these nucleotides serve dual roles: they are essential housekeeping metabolites central to core cellular processes like DNA/RNA synthesis and energy metabolism, while simultaneously acting as key second messengers regulating bacterial anti-phage defense systems (e.g., CBASS, Thoeris, Pycsar)[1-4]. Beyond signaling, the chemical identity of the genome itself is mobilized for defense; for example, Restriction-Modification (R-M) systems utilize site-specific DNA methylation to distinguish "self" from "non-self" DNA, providing a primitive yet robust epigenetic barrier against viral entry[5]. We previously established Bacmethy[6], a bioinformatic framework to characterize and analyze methylation patterns across bacterial genomes This co-option of core metabolic molecules for immune signaling creates unique vulnerabilities that phages exploit and that defenses must safeguard. For instance, phages like PaoP5 hijack the host's housekeeping c-di-GMP network not only to suppress immunity but also to promote virion assembly[7]. This pervasive use of nucleotides—spanning metabolism, signaling, and immunity—positions them as the central "chemical language" governing phage-host conflict and coevolution[8,9].

Despite rapid progress in identifying nucleotide-activated immune systems, a coherent understanding of how housekeeping nucleotide metabolism interfaces with defensive signaling remains limited. Bacteria repurpose core nucleotides and their derivatives—including cyclic dinucleotides, NAD^+, and non-canonical

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nucleotides such as dITP—into potent immune signals that activate diverse innate defense pathways. At the same time, phages have evolved highly specific countermeasures that target these nucleotide-based signaling axes, including sequestration of immune messengers by "sponge" proteins (e.g., Acb1, Tad1)[4,10,11], enzymatic degradation of signaling molecules, and interference with their biosynthesis or downstream function. These interactions highlight an underexplored layer of molecular conflict centered on nucleotide flux, specificity, and spatiotemporal control during infection.

Several recent reviews have advanced specific aspects of phage–bacteria interactions and nucleotide-based defense. Some works synthesize molecular mechanisms of nucleotide immune signaling systems such as CBASS, Pycsar, Thoeris, and type III CRISPR, highlighting common themes in how cyclic second messengers activate downstream effectors and comparing strategies phages use to evade these defenses[12,13]. Other reviews focus specifically on cyclic nucleotide signaling in phage defense and counter-defense, emphasizing key pathways, signaling molecules, and effector functions across systems using nucleotides as second messengers[14]. Additionally, other recent reviews systematically summarize phage–host interaction dynamics, including coevolutionary processes, arms-race and fluctuating selection mechanisms, and the diverse counter-strategies phages use to evade or overcome bacterial antiphage defense systems, providing broader context for signaling and ecological dynamics of phage–host interactions[15,16]. Although each of these works enriches a piece of the complex picture, they largely treat immune signaling mechanisms, nucleotide metabolism, and phage counter-adaptive strategies as modular topics rather than as an integrated, nucleotide-centric conflict. As a result, a synthesis that explicitly centers on nucleotides as a shared molecular currency linking metabolism, innate immunity, and phage counteradaptation is still lacking.

In this review, we focus on the critical intersection between housekeeping nucleotide metabolism and nucleotide-activated innate immune signaling in phage–bacteria interactions. We address three core questions structured to mirror our analysis: (1) Bacterial Defense Mechanisms (Section 2): How do bacteria transform core nucleotides and their derivatives (e.g., cyclic dinucleotides, NAD^+, non-canonical nucleotides like dITP) into signals that activate diverse immune pathways (CBASS, Thoeris, Pycsar, Kongming)? (2) Phage Counteradaptation Strategies (Section 3): How do phages precisely target these nucleotide-based signaling cascades? We examine phage-encoded "sponge" proteins (e.g., Acb1, Tad1) that sequester immune signals[4,10,11]. Nucleases that degrade them, and enzymes that sabotage their synthesis or function (e.g., modulating c-di-GMP[7], exploiting kinases like in Kongming to generate dITP for host defense[17]. (3) Knowledge Gaps and Translational Potential (Section 4): What critical unknowns persist regarding signal transduction specificity, evolutionary trade-offs in maintaining these defenses, and the spatiotemporal dynamics of nucleotide signaling during infection? How can understanding these nucleotide-centric interactions inform phage engineering and novel antimicrobial strategies?

By integrating structural, evolutionary, and mechanistic perspectives, this review synthesizes recent advances to provide a cohesive framework centered on nucleotides as the pivotal mediators of phage-bacteria warfare. We focus exclusively on nucleotide-activated innate immune systems and their evasion. While CRISPR-Cas represents adaptive immunity[18-20], it is included only where directly relevant to cyclic oligonucleotide signaling (e.g., Type III CRISPR-cOA[21,22]).

2 Nucleotide-activated immune systems: molecular sentinels of bacterial immunity

Bacterial nucleotide-activated immune systems share a conserved operational framework (Fig. 1 and Table 1): (1) pathogen sensing, (2) nucleotide second messenger synthesis, (3) effector activation, and (4) execution of abortive infection. These systems—including Pycsar, Thoeris, Type III CRISPR-Cas, CBASS, and Kongming—leverage cyclic nucleotides or NAD^+ derivatives as molecular alarms to trigger altruistic cell death or dormancy, thereby protecting clonal populations from phage predation[2-4]. This unified signaling logic positions nucleotide metabolism as the central axis of prokaryotic antiviral defense.

2.1 Pycsar: a cCMP/cUMP-dependent membrane attack complex

The cyclic nucleotides 3′,5′-cyclic cytidine monophosphate (cCMP) and 3′,5′-cyclic uridine monophosphate (cUMP), detected initially in human cells and previously associated with early embryonic development (cCMP)[23] and pro-apoptotic cell death (cUMP)[24], have been identified as essential second messengers in the Pycsar (short for pyrimidine cyclase system for antiphage resistance)[25] bacterial anti-phage defense system. Pycsar represents a recently discovered bacterial immune mechanism against phage predation, characterized by a novel cyclic nucleotide signaling pathway mediated by pyrimidine cyclase (PycC)[13,25,26]. Upon phage infection (e.g., T5 or T7), phage capsid proteins trigger PycC activation, which catalyzes the conversion of CTP/UTP into cyclic pyrimidine nucleotides cCMP/cUMP[27] as secondary messengers. These molecules exhibit a marked concentration increase within 15-40 minutes post-infection, specifically activating two classes of effector proteins[25]: the transmembrane protein PycTM induces membrane destabilization to initiate cell lysis, while the TIR domain-containing PycTIR forms oligomers with NADase activity, rapidly depleting cellular NAD^+ pools to induce metabolic collapse[26]. This dual mechanism collectively prevents phage propagation by inducing abortive infection. Notably, the Pycsar system demonstrates precise substrate selectivity (e.g., Y50/R97 residues in BcPycC determine cUMP specificity) and spatiotemporal regulation to minimize interference with host basal metabolism. Although certain phages evade defense through capsid protein mutations, such adaptations often confer replicative disadvantages in non-Pycsar hosts, reflecting an evolutionary trade-off.

2.2 Thoeris: NAD^+ depletion as a phage kill switch

Complementing pyrimidine-based defenses, the Thoeris system exemplifies a nucleotide-mediated antiviral paradigm conserved across domains of life[28]. Central to its defense mechanism is the Toll/interleukin-1 receptor (TIR) domain protein ThsB, which senses phage invasion and synthesizes cyclic ADP-ribose isomers (e.g., 1′′-3′ glycocyclic ADPR) via NAD^+ cleavage[4]. These signaling molecules specifically activate the effector ThsA, a bifunctional SIR2 (silent information regulator)-SLOG (SMF/DprA-LOG) protein, by binding its C-terminal SLOG domain to induce oligomerization and unleash its NADase activity. This enzymatic cascade depletes intracellular NAD^+ within minutes, triggering metabolic collapse and abortive infection before phage replication completes. Notably, Thoeris employs modular specificity: diverse ThsB paralogs (e.g., in

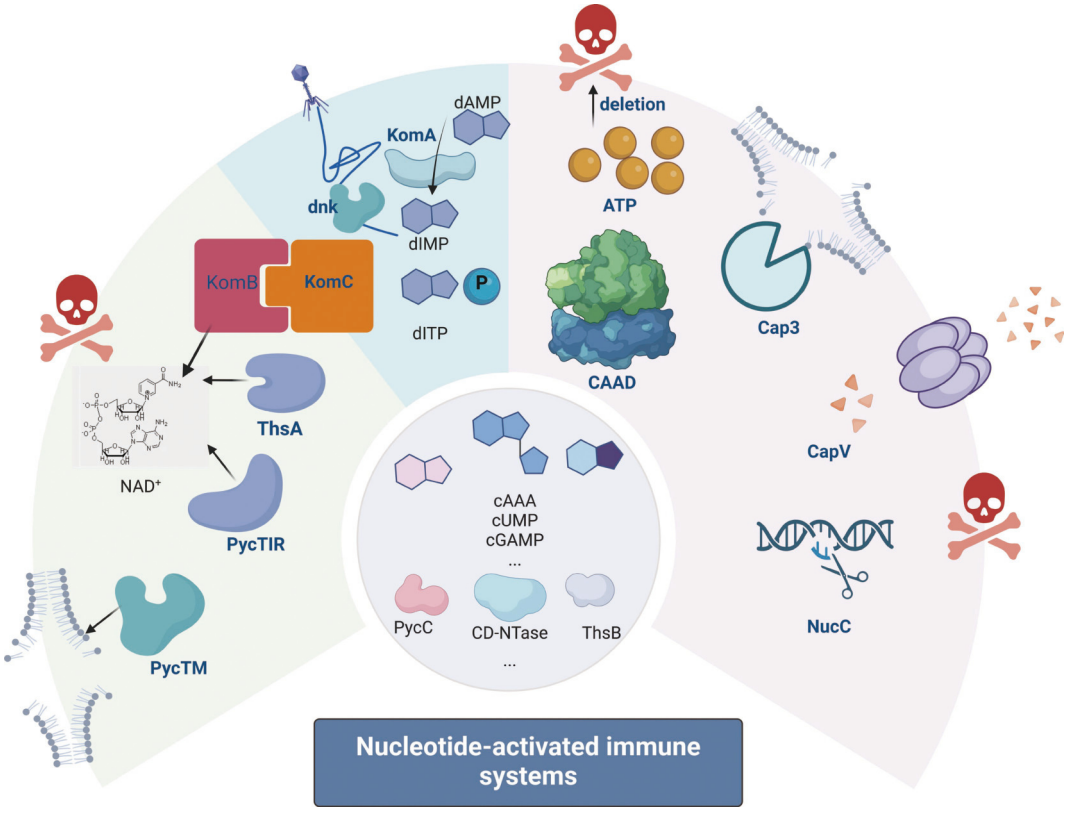

Figure 1 Nucleotide signaling-mediated multiple phage resistance systems. In the Pycsar system, PycC generates cCMP/cUMP as secondary messengers, which specifically activate PycTM to induce membrane destabilization and initiate cell lysis, or trigger PycTIR to rapidly deplete cellular NAD^+ pools, causing metabolic collapse and abortive infection. The Thoeris system involves ThsB producing 1′′-3′ glycocyclic ADPR to activate ThsA, which depletes intracellular NAD^+ and induces metabolic collapse with abortive infection before phage replication completes. The Type III CRISPR-Cas system detects cOA to deplete cellular ATP, leading to abortive infection. The CBASS system senses cyclic oligonucleotide signals to activate diverse effectors (Cap3 disrupts cell membrane, CapV creates membrane pores, NucC cleaves genomes) for abortive infection initiation. The Kongming system utilizes phage dnk kinase to phosphorylate endogenous dIMP into dITP, activating the KomB/C complex to rapidly deplete cellular NAD^+ pools and induce abortive infection.

Table 1 Comparative analysis of nucleotide-activated immune systems.

System	Signaling Molecule	Effector Mechanism	Phage Countermeasure	Taxonomic Range
Kongming	dITP	KomBC-mediated NAD^+ depletion	Dmp degrades dAMP precursor	Proteobacteria
CBASS	3′,3′-cGAMP etc.	Phospholipase/nuclease activation	Acb1, Acb2 sequesters cyclic nucleotides	Widespread
Thoeris	ADPR	SIR2-dependent NAD^+ hydrolysis	TirA mimics NAD^+ precursors	Archaea/Bacteria
Pycsar	cCMP/cUMP	PydA pore formation	Not yet reported	*Bacillus* spp.
Type III CRISPR-Cas	cAn	nucleases, proteases or adenosine deaminase, ATP hydrolysis	Acb1	*E.coli*

Bacillus species) recognize distinct phage-derived patterns, while a single ThsA effector can pair with multiple ThsB sensors to broaden antiviral coverage[29]. However, *E. coli* Thoeris defense systems with TM-macro (transmembrane and macro domain) ThsA effector utilize a distinct set of nucleotides for defense activation[30]. Strikingly, this TIR-catalyzed nucleotide signaling mirrors that of plant immune systems, where in which TIR-containing NLR proteins generate 2′ /3′ -cADPR or similar molecules to activate downstream resistance[31,32]. Manik et al. demonstrated that cADPR isomers serve not only as signaling molecules but also as critical intermediates in the synthesis of previously uncharacterized nucleotides associated with plant immunity. Furthermore, the research elucidated the cyclization sites of these cADPR isomers and delineated their production via TIR domains, as well as their signaling roles in both bacterial and plant immune pathways[33]. Rousset et al. investigated a bacterial immune system (type IV Thoeris) involving a TIR-domain protein and a caspase-like protease that defends against phage infection[34,35]. Such evolutionary convergence underscores primordial origins of nucleotide second messengers in immunity. Collectively, Thoeris not only redefines prokaryotic antiviral strategies through metabolic sabotage but also bridges mechanistic principles between bacterial

and eukaryotic immune networks, offering a framework for engineering synthetic defense systems or phage-resistant industrial strains.

2.3 Type III CRISPR-Cas: the most abundant immune systems of prokaryotes

Beyond dedicated nucleotide systems, Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) loci and CRISPR-associated (CAS) genes function as an adaptive immune system in bacteria and archaea to defend against invasive genetic elements[36], such as plasmids[37] and bacteriophages[38]. The palindromic repeats, interspersed with sequences known as spacers, are acquired from the genomes of invaders during infection[38]. Type III CRISPR-Cas systems represent a sophisticated adaptive immune mechanism in bacteria[39] and produce cyclic oligoadenylate second messengers[21,22] that combats phage invasion through a unique signaling cascade. Upon detecting phage-derived nucleic acids, these systems employ a Cas10-containing complex to synthesize cyclic oligoadenylate (cOA) second messengers[40], such as cyclic tetra-AMP (cA4) or cyclic hexa-AMP (cA6)[41-43], from ATP substrates. These cyclic nucleotides act as signaling molecules that allosterically activate ancillary effector proteins, such as nucleases[44], proteases[45,46] or adenosine deaminase[43], which execute antiviral responses. For instance, activated effectors may degrade viral DNA/RNA or induce abortive infection by depleting essential metabolites (e.g., ATP)[43] or disrupting membrane integrity[47], thereby halting phage propagation. This signal amplification strategy allows rapid system-wide immunity responses, as minimal phage detection triggers massive cOA production to ensure timely effector activation.

2.4 CBASS: cyclic nucleotide-triggered cell lysis

Operating through analogous nucleotide logic, the Cyclic Oligonucleotide-based Antiphage Signaling System (CBASS) represents a sophisticated bacterial innate immune mechanism that employs nucleotide second messengers to orchestrate collective defense against phage invasion[1,2]. Central to this system is a cGAS/DncV-like nucleotidyltransferase (CD-NTase)[48], which senses phage-derived DNA and catalyzes the synthesis of cyclic oligonucleotides[1,49] (e.g., 3',3'-cGAMP, c-di-AMP) from ATP or GTP substrates. These cyclic nucleotides act as second messengers that bind and activate downstream effector proteins, triggering programmed cell death or growth arrest in infected bacteria to abort phage replication[1]. For example, phospholipases (e.g., CapV) degrade bacterial membranes upon cGAMP binding[1], SAVED-domain nucleases (e.g., Cap4) induce genome fragmentation[50], while a class of bacterial CBASSs with associated HORMA and Trip13-like regulators and the nuclease effector NucC causes suicide by clipping the bacterial genome[51]. This "altruistic suicide" mechanism protects the bacterial population by eliminating compromised individuals before phages complete their lytic cycle. Notably, CBASS exhibits evolutionary parallels with eukaryotic immune pathways (e.g., cGAS-STING), where nucleotide signaling amplifies threat detection into a systemic response[52,53]. Recent structural studies reveal that bacterial E2 enzymes in some CBASS subtypes regulate CD-NTase activity through post-translational modifications, mimicking ubiquitination cascades to enhance cGAMP production[54]. What's more, eukaryotic synthases are activated by long, unmodified dsRNA in a sequence-independent manner[55], whereas prokaryotic type I CBASS system cyclases require a specific phage-derived RNA with special properties that distinguish it from other host-derived transcripts to avoid the induction of autoimmunity[56].

2.5 The Kongming system: a base modification-dependent defense pathway

Expanding the nucleotide defense repertoire, the recently discovered Kongming anti-phage defense system exemplifies a novel nucleotide-mediated immune signaling paradigm in bacteria[17]. This three-component system (KomA-KomB-KomC) employs base-modified nucleotides as signaling molecules through an ingenious "borrowing" mechanism: KomA (adenosine deaminase) converts host-derived dAMP to dIMP, which is subsequently phosphorylated by phage-encoded nucleotide kinases (DNKs) to generate the non-canonical nucleotide dITP, which is different from previously characterized immune signaling pathways using cyclic (oligo) nucleotides and ADPR-derived signals[1,4,21,22,47,49,57-59]. This pathogen-derived enzymatic activity, originally evolved for phage genome replication, is co-opted by the host to generate immune signals, exemplifying a tactical wisdom akin to the ancient Chinese military stratagem of "Zhuge Liang's Straw Boats Borrowing Arrows" (from which the system's nomenclature originates). This evolutionary interception mirrors the legendary resourcefulness of the Three Kingdoms strategist Zhuge Liang (Kongming), who famously repurposed enemy resources for defensive purposes - here, the host commandeers phage-encoded nucleotide kinases to transform viral metabolites into self-sacrificial alarm signals. Such molecular "borrowing" epitomizes the conceptual parallelism between prokaryotic immune innovation and classical Chinese military philosophy, where adversaries' intrinsic capabilities are subverted to orchestrate population-level defense. The dITP molecules specifically activate the KomB-KomC effector complex, triggering NAD^+ depletion through KomC's SIR2-like NADase activity and executing altruistic cell death to protect bacterial populations.

Silent information regulator 2 (Sir2) proteins mediate multiple phage defense systems by protecting against phage infection through NAD^+ depletion, thereby inhibiting the growth of infected cells. Examples include defense-associated sirtuin 2 (DSR2)[60], the prokaryotic Argonaute immune system (pAgo)[61], and the Sir2-domain-associated short prokaryotic Argonaute (SPARSA) system[62]. Recent studies have revealed that under uninfected conditions, intracellular ATP suppresses the enzymatic activity of Sir2. However, in *Staphylococcus aureus*, the Sir2-HerA complex detects phage infection by sensing a reduction in cellular ATP levels, thereby unleashing Sir2 NADase and nuclease activities to eliminate infected cells. This activation mechanism involves structural rearrangements where HerA binding induces conformational changes in Sir2, particularly the displacement of the α15 helix, thereby exposing the catalytic site for NAD^+ hydrolysis[63].

In addition to these nucleotide signaling molecule-mediated phage resistance systems, there exist nucleotide signal-regulated interactions between phages and bacteria (Fig. 2). Liang et al. discovered that the phage PaoP5 protein Dap1 exhibits dual functionality: it simultaneously targets the host protein DipA to modulate intracellular c-di-GMP levels in *Pseudomonas aeruginosa* PAO1, thereby influencing bacterial motility, while also acting on the phage HNH protein to facilitate virion assembly[7]. Notably, function loss of Dap1 significantly impacts phage therapeutic efficacy[7]. Similarly, phage PB1 encodes YfiN-interacting peptides (YIPs) that engage with *Pseudomonas aeruginosa* YfiN, altering c-di-

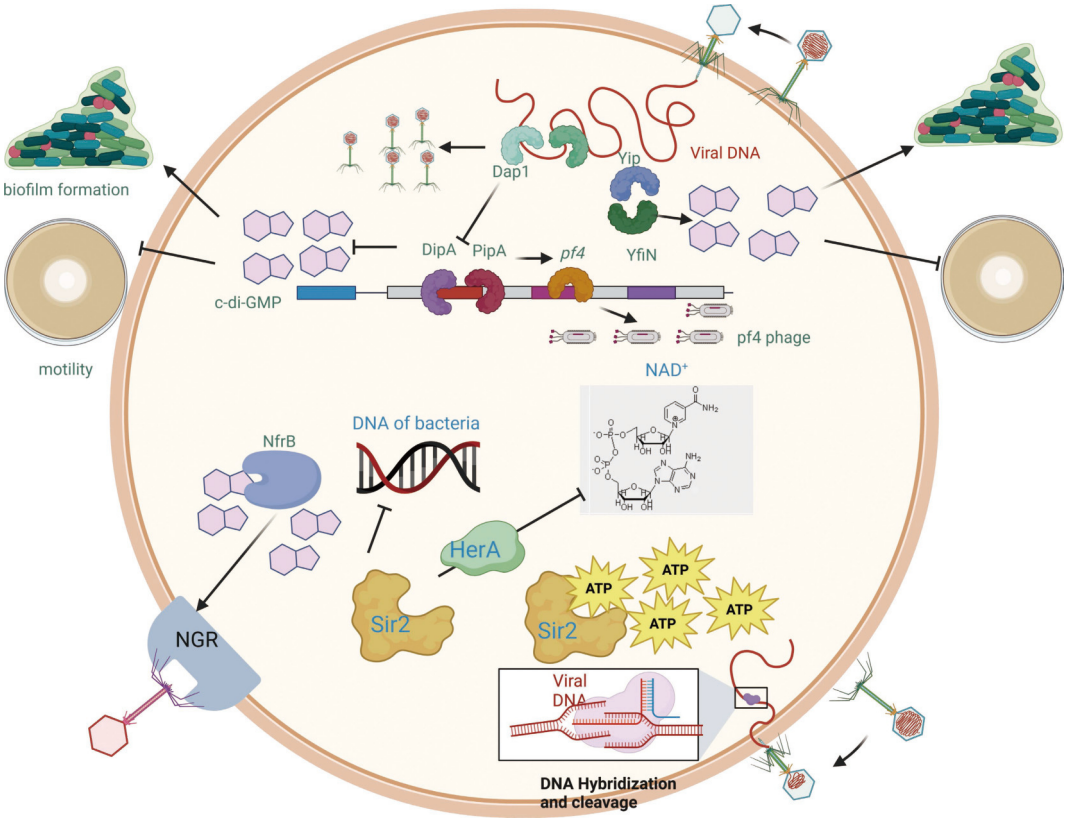

Figure 2 Nucleotide signaling also mediates interactions between phages and their hosts. The phage-encoded proteins Dap1 and Yip interact with the *Pseudomonas aeruginosa* c-di-GMP phosphodiesterase DipA and synthase YfiN, modulating c-di-GMP levels to influence bacterial biofilm formation and motility. Additionally, Dap1 plays a crucial role in phage assembly. The c-di-GMP metabolic enzyme PipA promotes the formation of the *Pseudomonas aeruginosa* filamentous phage Pf4. NfrB binding to c-di-GMP is critical for the infection of phage N4. In the HerA-Sir2 system, ATP inhibits Sir2 activity. Upon phage infection, when ATP levels are rapidly depleted, Sir2 becomes active and swiftly depletes cellular NAD^+ pools.

GMP homeostasis to reduce bacterial motility and enhance biofilm formation[64]. Furthermore, the c-di-GMP phosphodiesterase PipA regulates production of *Pseudomonas aeruginosa* Pf4 phage[65]. In *E. coli* K12, the c-di-GMP-binding protein NfrB is essential for successful infection by phage N4[66], which employs a novel surface glycan (NGR) as its host receptor. This infection process is modulated by the second messenger c-di-GMP, with N4 infectivity being specifically potentiated by the diguanylate cyclase DgcJ, while the phosphodiesterase PdeL effectively protects *E. coli* from N4-mediated lysis[67]. These findings collectively demonstrate the widespread and critical regulatory roles of oligonucleotide signaling molecules in phage-host interactions.

3 Phage countermeasures against nucleotide-mediated immunity

Phage counterstrategies universally target nucleotide signaling cascades through three primary mechanisms: enzymatic degradation of immune messengers, molecular sequestration of cyclic nucleotides, and spatial evasion of detection systems (Fig. 3). The evolutionary arms race between phages and bacterial immune systems has driven the emergence of specialized phage proteins that disrupt cyclic nucleotide signaling-the central pillar of prokaryotic antiviral defense. These countermeasures fall into two overarching strategies: enzymatic degradation and molecular sequestration of immune signaling molecules. A central tactic involves enzymatic degradation of immune alarmones. For instance, T4 phage encodes "molecular scissors" (Acb1[10,68]), a metal-independent hydrolase that selectively cleaves both CBASS-derived 3′, 3′-cyclic dinucleotides and Type III CRISPR-Cas-produced cyclic trinucleotides (e.g., cAAA), effectively silencing NucC[51] and Cas10[50] effector activation. This dual-substrate specificity allows Acb1 to suppress disparate immune pathways through a shared mechanism, with its activity timed to late infection stages to avoid premature host lysis. Similarly, SBSphiJ phage deploys Apyc1[10], a zinc-dependent metalloenzyme that linearizes pyrimidine-based cyclic mononucleotides (cCMP/cUMP[27]), thereby blocking Pycsar-mediated NADase-driven abortive infection. The rapid diversification of these enzymes-Acb1 homologs share as little as 25% sequence identity across phages[10] -mirrors the hypervariability of bacterial immune systems, underscoring a coevolutionary tug-of-war.

Beyond enzymatic sabotage, phages employ molecular "sponges" to sequester cyclic nucleotides with remarkable specificity. The hexameric Tad1[11] protein, exemplified by phiKZ phage's gp184, neutralizes Thoeris-derived 1″-3′-gcADPR and CBASS cyclic trinucleotides through spatially segregated binding interfaces[10], while its paralog Tad2[69] captures both gcADPR and CBASS dinucleotides via dual ligand-binding clefts[70]. These proteins achieve cross-system suppression by exploiting modular architectures: Tad1 dedicates distinct domains to gcADPR and cyclic nucleotides, whereas Tad2 utilizes inter-subunit flexibility to

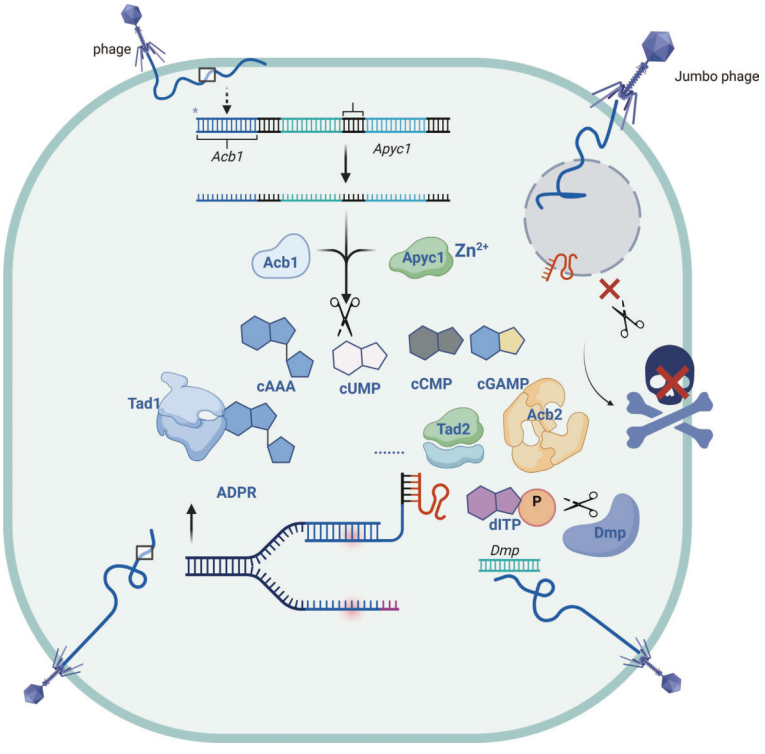

Figure 3 Phages counteract phage defense systems through diverse mechanisms. Acb1 and Apyc1 act as "molecular scissors" that cleave signaling molecules to evade host defense systems. Tad1, Tad2, and Acb2 function as "molecular sponges," sequestering signaling molecules to escape detection. In the anti-Kongming system, phages encode Dmp to degrade dITP, thereby counteracting host defense.

accommodate diverse ligands. Such versatility extends to Acb2[71], a broad-spectrum scavenger that simultaneously binds cyclic trinucleotides (cA3) and dinucleotides, blocking phospholipase CapV and nuclease NucC across CBASS subtypes[72]. The genetic clustering of these countermeasures in phage "anti-defense islands"-often alongside methyltransferases-reflects their coordinated evolution to bypass multilayered immunity.

Spatial evasion strategies further exemplify phage ingenuity. Jumbo phages (genomes> 200 kb)[73] PCH45 construct a proteinaceous nucleus to shield DNA from Type I/II CRISPR-Cas nucleases. However, this physical barrier is circumvented by RNA-targeting Type III CRISPR-Cas systems, which detect viral mRNA exported to the cytoplasm for translation[74]. Upon transcript recognition, Cas10 synthesizes cyclic oligoadenylates (cA3/cA4), activating collateral nucleases like NucC (DNase) or Csm6 (RNase) to degrade host DNA/RNA and induce abortive infection[44]. This mechanism exploits the unavoidable exposure of phage transcripts-a vulnerability inherent to transcriptional cycles-while bioinformatic studies reveal widespread coupling of Type III systems with diverse nucleases, highlighting their evolutionary adaptation to nucleus-forming phages.

The conflict extends to nucleotide metabolism itself. T5 phage counters host defenses by expressing Dmp phosphatases that degrade dITP[17], a precursor of mutagenic dXTP nucleotides used by bacterial systems (e.g., Dnd[75,76], Gabija[77]) to disrupt phage DNA. Hosts retaliate through dual-substrate conversion pathways, maintaining defense efficacy despite phage interference. Concurrently, the discovery of Kongming systems has unveiled base-modified nucleotides (e.g., 2′-O-methylated cGAMP analogs) as stealthy immune messengers resistant to phage hydrolases[17]. These molecules activate effector nucleases through distinct recognition mechanisms, forcing phages to evolve proteases or nucleases targeting Kongming components—an escalation illustrating the infinite creativity of nucleotide-centric warfare.

Collectively, phage countermeasures prioritize intercepting upstream signaling molecules over downstream effectors-a strategic choice minimizing genomic investment while maximizing immune suppression. This paradigm is evolutionarily conserved, as evidenced by the ubiquity of Acb1-like hydrolases and Tad1-like sponges across phage genomes. Biotechnologically, these mechanisms offer dual promise: Acb1's programmable nucleotide degradation could modulate human cGAS-STING signaling[10,78], while Kongming-inspired molecules may engineer pathogen-specific immune activators. By subverting nucleotide-mediated alarm systems, phages not only exemplify nature's molecular ingenuity but also provide blueprints for manipulating immune pathways across life's domains.

4 Biotechnological and medical applications

4.1 Phage therapy

The resurgence of phage therapy has demonstrated significant clinical breakthroughs in combating multidrug-resistant *Acinetobacter baumannii* (MDR-AB) (Fig. 4b). In 2024, Qu et al. from Shenzhen Third People's Hospital reported a case of extensively drug-resistant *A. baumannii* (XDR-AB) pulmonary infection successfully treated with inhaled phage therapy. Post-treatment analysis revealed a dramatic reduction in bacterial load and a surge in phage DNA abundance[79]. Phage Spe5P4 also has obvious therapeutic effect on pulmonary infection caused by multidrug-resistant *Serratia marcescens*[80]. A landmark clinical

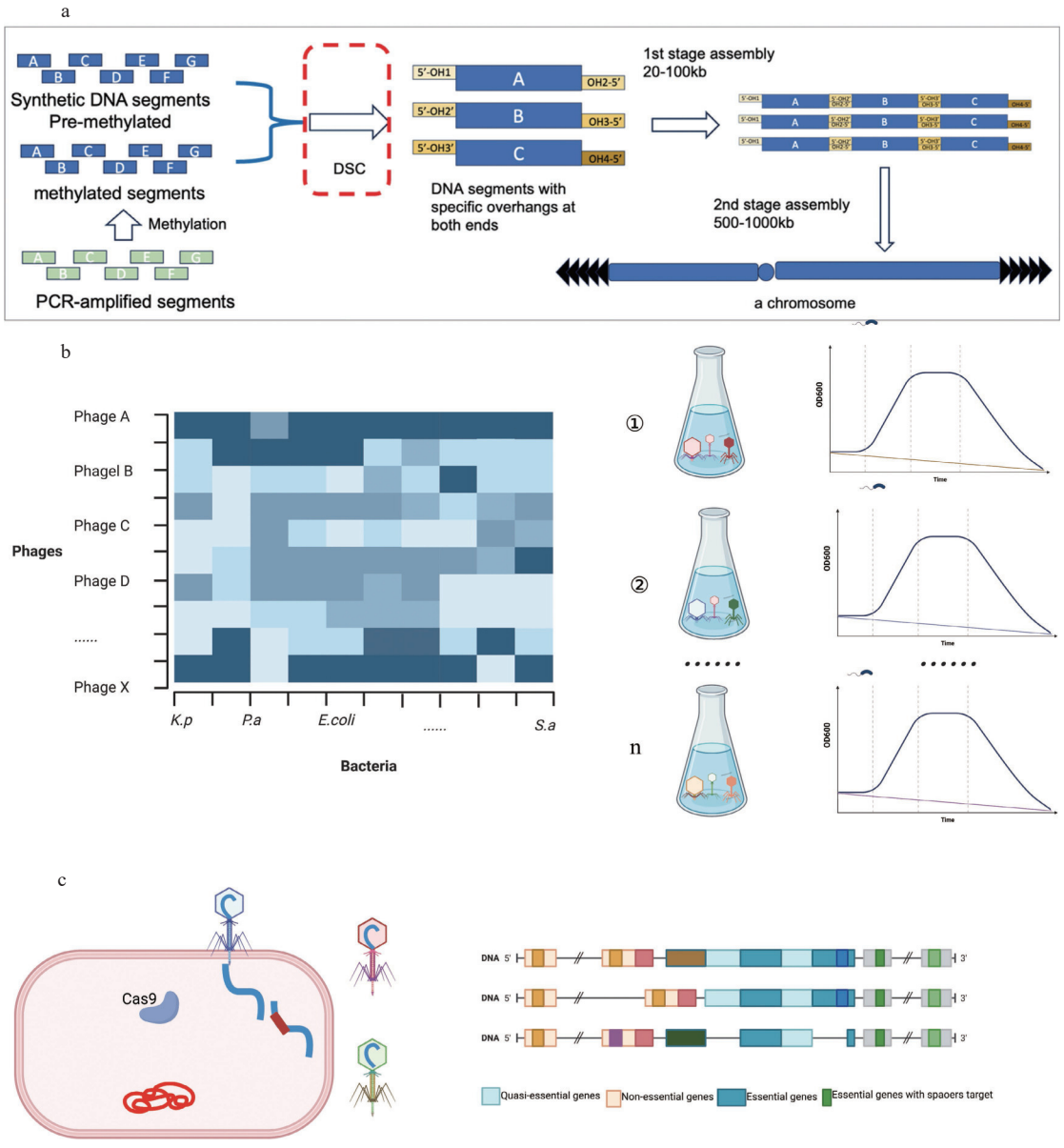

Figure 4 Applications of phages. (a) De novo synthesis of phages via artificial PCR: Ideal phages can be directly designed through artificial genome assembly and *E. coli*-based amplification. (b) Broad-spectrum phage cocktails for therapy: phage lytic activity testing against hosts enables the synthesis of tailored cocktails for therapeutic use. (c) Genome streamlining via CRISPR: Non-essential genes are removed using CRISPR-based systems to create artificially streamlined phage genomes while maintaining high lytic efficiency.

study published in 2019 further demonstrated the feasibility of phage therapy by successfully treating *Mycobacterium abscessus* infections using a cocktail of genetically engineered phages[81]. In 2025, Fudan University Pediatric Hospital achieved the first pediatric cure using nebulized phage cocktails, eliminating pathogens in an 11-year-old patient within two weeks without adverse effects. Extending this concept toward clinical scalability, Subedi et al. reported the use of a rationally designed, hospital-specific phage cocktail to treat *Enterobacter cloacae* infections, achieving coverage of 88% of the hospital's clinical isolates[82]. These clinical applications underscore the feasibility of precision phage screening and tailored delivery protocols, while simultaneously highlighting the critical importance of deciphering bacterial resistance mechanisms to phages. In 2024, Wang et al. analyzed phage-host interaction data and constructed an engineered phage library with exchangeable receptor-binding proteins (RBPs). This innovation successfully altered the capsular specificity of *Klebsiella pneumoniae* (e.g., redirecting it from KL2 to KL57)[83], establishing a standardized platform for targeted therapy against drug-resistant bacterial infections.

4.2 Synthetic biology integration

Advances in synthetic biology help expand the application of bacteriophages. (Fig. 4a and 4c). Synthetic biology approaches have enabled engineered phages to broaden their host range, introduce additional functions, and modulate antimicrobial efficiency[84]. For example, a phage chassis can be reconfigured by swapping tail components to retarget infection toward diverse hosts such as *E.*

coli, *Yersinia* and *Klebsiella* by swapping the tail components on the chassis of T7 phage[85] and host-range determining regions (HRDRs) within structural proteins have been mapped to enable high-throughput generation of mutant libraries with expanded host specificity[86]. Phages have also been engineered as reporter platforms by fusing capsid proteins with fluorescent proteins or chemical labels, allowing specific bacterial populations to be detected and visualized in complex environments[87]. Beyond targeting and detection, synthetic biology–driven engineering has enhanced phage antimicrobial capabilities by equipping phages with heterologous payloads—such as biofilm-degrading enzymes[88], quorum-quenching proteins[89], antimicrobial peptides[90,91], or toxins—that disrupt bacterial defenses and increase killing efficiency beyond natural lytic activity, offering versatile strategies to combat multidrug-resistant pathogens and complex biofilms[92,93]. Synthetic biology has also been used to refine phage genomes themselves; iterative genome reduction systems based on CRISPR-Cas9 have streamlined phage genomes by removing non-essential elements while retaining high lytic efficiency, illustrating how precise genome editing can optimize phage traits for therapeutic use[94]. While synthetic biology enriches the application of phages, bacteriophages also act as a powerful tool in synthetic biology. Phage display technology enables the presentation of diverse peptides or proteins on phage surfaces for high-throughput screening and functional selection platforms[95]. Phage-derived parts also empowers the synthetic biology tool box like genetical expression[96] and bacterial genome manipulation[97,98]. These combined advances position engineered phages as programmable biological agents for targeted antimicrobial, diagnostic, and delivery applications.

4.3 Microbiome sustainability

Gut phages play a critical role in regulating microbiome structure and function (Fig. 5c and 5d). In the study by Ma et al., a large-scale cultivation approach enabled the isolation and characterization of 209 non-redundant phages targeting 42 human gut commensal bacterial species (15 Bacteroides and 19 Firmicutes, among others) named GPIC (gut phage isolate collection), laying the foundation for precise modulation of gut microbiota through phage cocktails[99]. Ruan et al. revealed that phage predation can promote conjugation-mediated plasmid transfer and proliferation by slowing spatial mixing of microbial populations during surface-associated growth, challenging the conventional notion that phage predation solely reduces microbial abundance. This demonstrates that phages not only directly influence microbial populations through host lysis but may also indirectly promote or suppress antibiotic resistance gene dissemination by reshaping microbial community spatial architecture[100]. Furthermore, research from Harvard Medical School uncovered a highly interactive and dynamic community where lytic phages coexist with and suppress targeted bacteria, with their effects propagating through other microbiome members to ultimately modulate the gut metabolome[101]. Collectively, these findings highlight the pivotal regulatory roles of phages in microbial ecosystems.

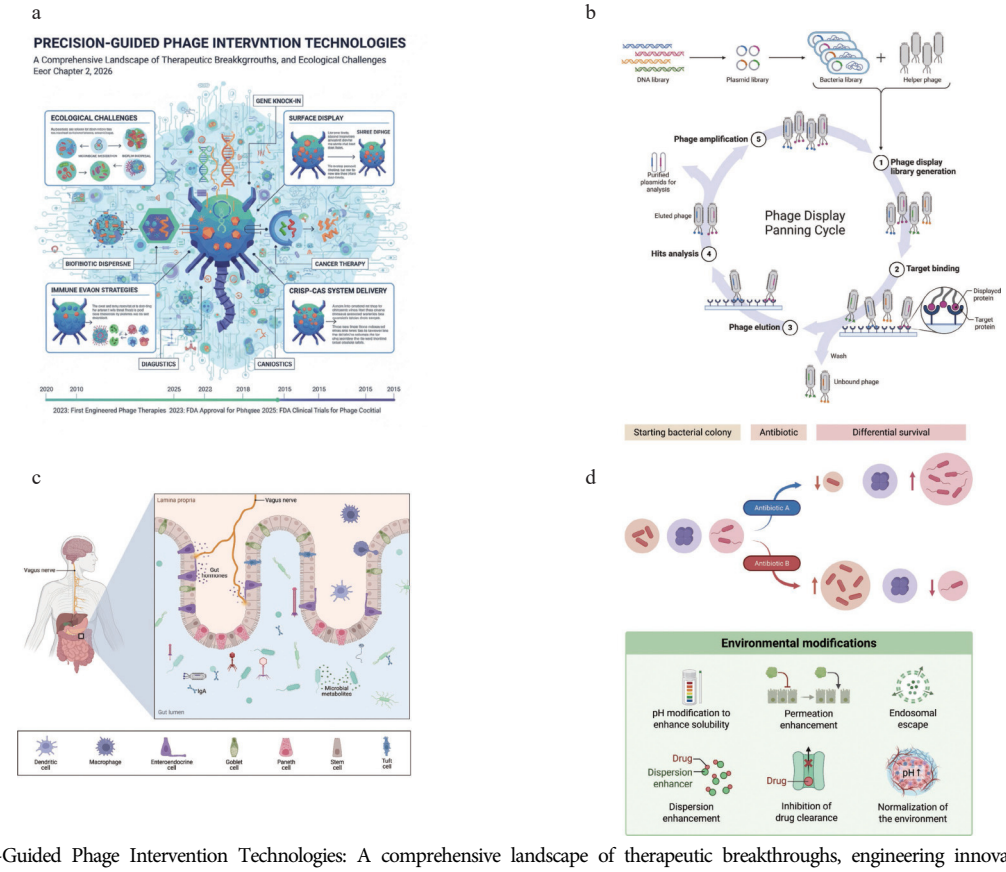

Figure 5 Precision-Guided Phage Intervention Technologies: A comprehensive landscape of therapeutic breakthroughs, engineering innovations, and ecological challenges. (a) An integrated framework of engineered phage technologies, spanning from genetic innovations and therapeutic applications to ecological integration and clinical milestones. (b) Phage display as a precision-guided engineering platform for selective ligand discovery and targeted applications. (c) Microbiome sustainability: The human body as a vast ecological reservoir of microbes. (d) Phage therapy illuminates solutions to antimicrobial resistance and ecological challenges.

4.4 Resistance mechanisms and ecological challenges

A. baumannii employs sophisticated genomic defenses against phages. Jilin University identified 21 "defense system hotspots" in its genome, including CRISPR-Cas and restriction-modification systems, with the SspBCDE system detected 2,203 times in the HS6 hotspot, acting as a primary barrier[102]. Prolonged phage exposure may trigger cross-resistance; Pan and colleagues from Shenzhen Third People's Hospital observed overexpression of the AcrAB-TolC efflux pump, which confers resistance to both phages and antibiotics like ciprofloxacin. Additionally, biofilm penetration remains a hurdle[103]. In 2024, Zheng et al. found that phage P1068 achieved >90% clearance of free *A. baumannii* but only ~50% efficacy against biofilm-embedded bacteria due to extracellular polymeric substance (EPS) barriers[104].

5 Summary

Nucleotide signaling molecules constitute the pivotal "molecular codes" governing the bacteriophage-bacteria arms race. Recent discoveries illuminate how these molecules integrate evolutionary conservation with mechanistic innovation. Exemplifying this duality, the Kongming system exemplifies this duality by co-opting phage-encoded kinases to convert dAMP into dITP—a noncanonical nucleotide that activates NAD^+ depletion to block phage replication, thereby embodying a 'borrowed arrows' strategic paradigm[17]. Conversely, phages deploy "sponge proteins" like Acb2, Tad1, and Tad2 to sequester cyclic nucleotides (e.g., CBASS-derived cGAMP and Thoeris-specific gcADPR), effectively neutralizing bacterial immune signals through precise molecular interception[11,69,71]. These reciprocal adaptations collectively underscore nucleotide metabolism as the central battlefield driving host-pathogen coevolution.

Technological convergence now provides unprecedented tools to dissect these intricate interactions. Single-cell biosensors resolve nucleotide flux dynamics at sub-second resolution during infection[105], while engineered LAMP primers incorporating artificial mismatch bases detect single-nucleotide mutations critical for phage escape[106]. Beyond temporal resolution, structural phylogenomics reveals deep evolutionary conservation between prokaryotic CBASS and eukaryotic cGAS-STING pathways, strongly implicating cyclic nucleotide signaling as a primordial immune principle[107]. Complementing this, long-read metagenomics uncovers extensive horizontal transfer of nucleotide mimicry domains in temperate phages, a mechanism enabling host-range expansion through structural innovation[108]. Together, these advances establish a multiscale framework for decoding nucleotide-centric warfare.

Building on these mechanistic and technological insights, Artificial Intelligence (AI) emerges as a transformative tool for therapeutic development. Multimodal neural networks integrate cryo-EM data and molecular dynamics simulations to map allosteric pockets in nucleotide-binding proteins (e.g., Tad1 sponges)[70], potentially enabling the rational design of anti-resistance peptides. Furthermore, integrating structural predictions with phage genomic data promises to accelerate the engineering of targeted antimicrobials.

Future progress demands systematic integration across three key domains: (1) Developing standardized nucleotide interactome databases to characterize unannotated defense systems; (2) Utilizing microfluidics-enabled coevolution studies to model nucleotide-driven resistance trajectories; (3) Establishing ethical frameworks governing phage-delivered editors for human metabolic engineering. Despite challenges in ecological risk mitigation, the confluence of fundamental nucleotide biology and synthetic engineering heralds an era of programmable control over microbial ecosystems, ultimately positioning nucleotide signaling as the cornerstone of next-generation antimicrobial strategies.

Looking ahead, a phage-centric engineering paradigm—guided by artificial intelligence—promises to transform phage therapy from a pathogen-reactive to a phage-predictive discipline(Fig. 6). By leveraging AI models trained on phage genomes, protein structures, and nucleotide signaling networks, we can rapidly design synthetic phages with tailored host ranges, enhanced immune evasion, and minimal genomic footprints. This approach shifts the focus from screening natural phages against bacterial infections to de novo programming of phage functions, enabling the development of personalized phage therapeutics within clinically actionable timeframes. The integration of nucleotide signaling insights with machine learning and synthetic biology will thus pave the way for next-generation antimicrobial platforms that are adaptive, precise, and resilient against resistance evolution.

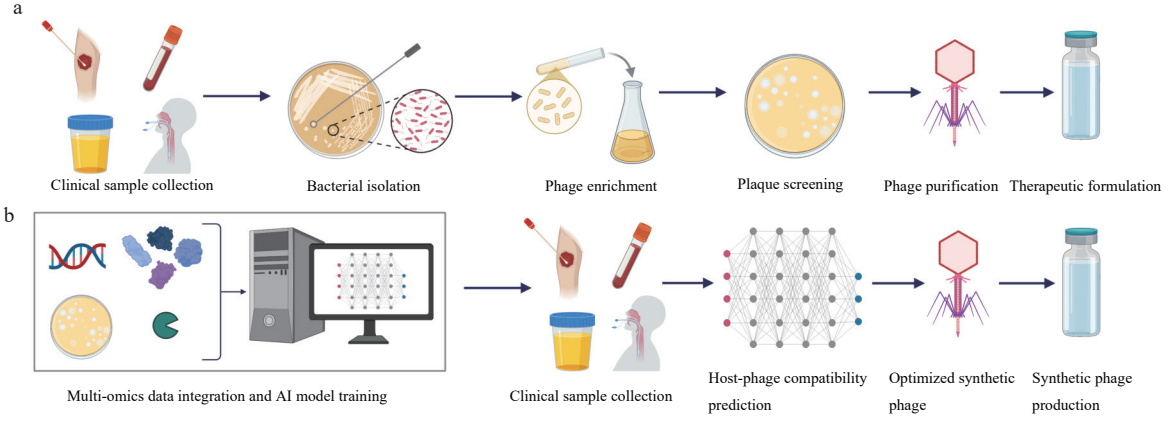

Figure 6 Future paradigms in phage engineering–guided by artificial intelligence–hold promise to transform phage therapy from a pathogen-responsive discipline into a proactive, predictive phage-based field. (a) Traditional phage therapy process. (b) Artificial intelligence-guided synthetic phage process. By leveraging AI models trained on phage genomes, protein structures, and nucleotide signaling networks, we can rapidly design synthetic phages with custom-designed host ranges, enhanced immune evasion capabilities, and minimal genomic footprints, thereby disrupting the traditional route of isolating suitable phages only after pathogenic bacterial infections occur.

Abbreviations

cNMPs	cyclic Nucleotide Monophosphates
c-di-GMP	Cyclic di-guanosine Monophosphate
c-di-AMP	Cyclic di-adenosine monophosphate
ppGpp	guanosine tetraphosphate
CBASS	Cyclic-Oligonucleotide-based Antiphage Signaling System
dITP	deoxyinosine triphosphate
CRISPR-Cas	Clustered regularly interspaced short palindromic repeat–CRISPR-associated systems
cOA	cyclic oligoadenylate
cCMP	3′,5′-cyclic cytidine monophosphate
cUMP	3′,5′-cyclic uridine monophosphate
Pycsar	pyrimidine cyclase system for antiphage resistance
PycC	pyrimidine cyclase
CTP	Cytidine Triphosphate
UTP	Uridine Triphosphate
cCMP	cyclic cytidine monophosphate
cUMP	cyclic uridine monophosphate
TIR	Toll/interleukin-1 receptor
ADP	Adenosine Diphosphate
TM-macro	transmembrane and macro domain
ADPR	Adenosine Diphosphate-Ribose
cADPR	Cyclic Adenosine Diphosphate-Ribose
CD-NTase	cGAS/DncV-like nucleotidyltransferase
dAMP	Deoxyadenosine Monophosphate
dIMP	Deoxyinosine Monophosphate
Sir2	Silent information regulator 2
DSR2	defense-associated sirtuin 2
pAgo	prokaryotic Argonaute immune system
SPARSA	Sir2-domainassociated short prokaryotic Argonaute
YIPs	YfiN-interacting peptides
CiPGr	CRISPR-Cas9 Iterative Phage Genome Reduction
GPIC	gut phage isolate collection

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Data available statement

N/A

Author contribution

J.D. conceptualized the work, visualized the figures and tables, and drafted the manuscript. M.W.C. and C.Y.G wrote and reviewed the manuscript. Z.C. reviewed the manuscript and provided funding for the work. L.Y. conceptualized the work, providing funding, wrote and reviewed the manuscript.

Ethics approval and consent

N/A

Consent for publication

All authors agree to publish.

Conflicts of interest

The authors declare no conflicts of interest.

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