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ARTICLE

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PHAGE THERAPY: THE ULTIMATE ALTERNATIVE AGAINST ANTIBIOTIC RESISTANCE (ABR) - CHALLENGES, ADVANTAGES, OPPORTUNITIES AND THE PIVOTAL ROLE OF DNA-BINDING PROTEINS IN STEERING MODERN STRATEGIES THROUGH LYTIC-LYSOGENIC CONTROL

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The relentless rise of antibiotic resistance (ABR) presents a grave threat to global health, steadily diminishing the effectiveness of conventional antibiotics against once-manageable infections. In 2023 alone, nearly two million lives were lost to antibiotic-resistant infections, with projections warning of a 70% increase in fatalities by 2050. This alarming trend underscores the urgent need for alternative therapeutic strategies.

In this review, we explore phage therapy as a promising solution. Although discovered long before antibiotics, early phage therapy efforts were hampered by limited scientific understanding, inadequate documentation, and challenges in application. However, today's advancements in genomics, molecular biology, and phage engineering have breathed new life into this strategy, paving the way for more effective therapeutic use.

Central to these innovations are the DNA-binding proteins within bacteriophages, which regulate the switch between lytic and lysogenic cycles, redefining phage-based treatments. Deciphering these proteins unlocks new therapeutic avenues, enabling precise control of phage life cycles to optimize bacterial eradication. Additionally, engineered phages offer the potential to deliver CRISPR-Cas systems, facilitating targeted gene editing to disable resistance genes or reduce bacterial virulence.

By harnessing the full potential of modern phage therapy, we stand at the brink of a revolution in precision medicine, equipped to outpace the adaptive evolution of ABR pathogens and restore control over drug-resistant infections.

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Introduction

Problem of Antibacterial Resistance

Antibiotic Resistance (ABR) is one of the most critical global public health problems threatening human existence in the current century**.** ABR occurs when bacteria acquire resistance to antibiotic therapeutics to which they were previously susceptible[1]. During World War II, antibiotics became available, and

their widespread use changed how humans fought the infection. They were called "wonder drugs" at the time due to their ability to safely and effectively treat infectious diseases. Their substantial use over the past 70 years has since saved countless lives and continues to do so today. However, with the overuse and misuse of these drugs in humans and animals, scientists are discovering a disturbing trend of antibiotic resistance among bacteria. Antibiotics are now no longer the "wonder drugs" they once were. As a consequence of this antibiotic resistance, it could cause a common bacterial infection creating severity and even leading to death. Increased use and misuse of antibiotics and other microbial stressors, such as pollution, create favourable conditions for bacteria like microorganisms to develop resistance[2]. For example, bacteria in water, soil and air can acquire resistance following contact with resistant microorganisms through gene transfer. The World Health Organization (WHO) lists ABR among the top 10 threats to global health, stating, "Antibiotic Resistance can affect people of any age, at any time and in any country" (WHO, 2018)[3]. At this moment, antibiotic resistance represents the most life-threatening disease. Anti-bacterial resistance threatens human and animal health, the environment, food and nutrition security and safety, economic development, and societal equity. Counting the severity of the increasing emergence of novel resistance compared to the extremely slow appearance rate of new antibiotics already raises the question in our mind: are we losing the battle? Is there a way out?

As an expected outcome of thought, expectance of the appearance of new antibiotics could not be ignored, but here comes the obvious: if that antibiotic goes for resistance too? Standing on this conjecture, this review could bring fresh wind. It explores the potential of one of the bacteria's oldest enemies, "the Phages".

Bacteriophages

Bacteriophages, or phages, are viruses that specifically target bacteria. Phage therapy involves using phages to treat bacterial infections. Phages are everywhere. From the soil to our guts, there are thousands of different types.

In contrast to many antibiotics, which obliterate harmful bacteria while simultaneously decimating the microbiota (thus triggering a new set of problems), each phage has evolved to target bacterial strains or species more narrowly. This specificity makes phage therapy an attractive alternative for managing infections, especially those caused by multi-drug resistant (MDR) bacteria[4].

Yet, phage therapy has existed mainly on the fringes of medicine, particularly in Western countries like the U.S., where it is occasionally approved for compassionate use (i.e., used on an emergency basis when no other approved therapies are available). Why? And what needs to happen to make phage therapy mainstream? The answers are entrenched in historical skepticism, regulatory and manufacturing hurdles, and physiological aspects of phages themselves. To understand this, we must appreciate phage therapy in detail.

Phage Therapy

Phage therapy is an alternative approach to combat AMR, utilizing bacteriophages and viruses that specifically infect and kill bacteria. There are several advantages because phage therapy evolved when antibiotics or other related therapies were not even available[5].

History of Phage Therapy: Although not fully understood then, the concept of phage therapy has roots in ancient civilizations where people observed the beneficial effects of using certain substances to treat infections[6]. Here are a few examples of early practices that can be considered precursors to phage therapy:

Ancient Egypt: Ancient Egyptian medical texts dating back to around 1550 BCE mention using moldy bread and other substances to treat infected wounds. It is conceptualized that these molds likely contained bacteriophages, as they effectively inhibited the growth of bacteria.

Ancient Greece: The Greek physician and father of modern medicine, Hippocrates (460-370 BCE), observed that certain waters from sacred wells and rivers had healing properties for various ailments. These waters, known as "healing waters", were believed to contain substances with antibacterial properties, including phages.

Folk Medicine Practices: Throughout history, folk medicine traditions worldwide utilized natural substances, such as honey, garlic, and certain herbs, to treat infections. Some of these substances likely contained phages, contributing to their antimicrobial effects.

The holy water of the river GANGES in India: In ancient India, it was believed that the Ganges water was pure and good for our health. Pilgrims used to collect the river water and store it in their households for days and months. It was believed that this water would not be contaminated because of the presence of phages in high concentrations in the river Ganges.

Félix d'Herelle: The formal recognition and understanding of phages as viral entities with therapeutic potential emerged in the early 20th century. The French-Canadian scientist Félix d'Herelle is credited with coining the term "bacteriophage" and conducting groundbreaking research on the therapeutic use of phages. In 1919, he successfully treated dysentery patients with phages, marking the beginning of modern phage therapy.

It is important to note that these ancient practices needed to understand bacteriophages or the mechanisms underlying their therapeutic effects comprehensively. Due to the absence of clear understanding and logical interpretation, there was a temporary decline in phage therapy[7].

Challenges in Phage Therapy: It took more work to explain the incidence of reduced interest in phage therapy as multiple reasons are behind it[8,9]. Some of them include,

Discovery of Antibiotics: One of the most significant reasons for the decreased popularity of phage therapy lies in the discovery and widespread use of antibiotics in the mid-20th century, revolutionizing medicine. As Paul Ehrich predicted, they were the required magic bullets to solve the problem of infectious diseases. Antibiotics were effective against many bacterial infections and were relatively easy to produce, store, and administer. As a result, antibiotics quickly became the go-to treatment for bacterial infections, overshadowing phage therapy[10].

Lack of Scientific Understanding: During the early days of phage therapy, the scientific understanding of bacteriophages still needed to be improved. The need for standardized experimental protocols, quality control measures, and a clear understanding of phage biology hindered the widespread adoption of phage therapy. On the other hand, Antibiotics, with their well-established mechanisms of action, were seen as more reliable and easier to use.

Phage Selection: This point is closely associated with the lack of phage biology and therapy knowledge. Identifying the appropriate phages that effectively target and kill the specific bacteria causing the infection can be complex.

Regulatory Challenges: The development of regulations and approval processes for phage therapy lagged behind those for antibiotics. The regulatory environment focused primarily on evaluating the safety and efficacy of chemical compounds rather than live viruses, making it challenging to navigate the approval process for phage-based treatments.

However, with all those above-mentioned challenges, it is important to note that phage therapy never completely disappeared. It continued to be practiced in some regions, such as Eastern Europe and the former Soviet Union, where it was researched and utilized throughout the 20th century[8].

Advantages of Phage Therapy: In recent years, there has been a global resurgence of interest in phage therapy due to the pressing issue of antimicrobial resistance and the need for alternative treatment options. But before discussing this, let us look at the advantages of phage therapy over others, especially Antibiotic therapeutics[9].

Specificity Factor: Bacteriophages are highly specific in their host range, meaning they can target and kill particular strains or species of bacteria while leaving beneficial bacteria and the human microbiota untouched. This specificity reduces the likelihood of developing resistance compared to broad-spectrum antibiotics.

Coevolution: Bacteriophages have coevolved with bacteria for billions of years, resulting in an ongoing arms race. Phages continually adapt and evolve to counter bacterial resistance mechanisms, ensuring their effectiveness against resistant strains.

Versatility: Phages can infect bacteria through various mechanisms, including direct lysis of bacterial cells or releasing enzymes that degrade bacterial cell walls. This versatility allows for different approaches to targeting and killing bacteria, potentially overcoming certain resistance mechanisms.

Self-replication: Bacteriophages can replicate within the host bacterium, increasing their numbers and potentially eradicating bacterial infections. This self-replicating ability reduces the need for repeated dosing, making phage therapy more cost-effective.

Combination Therapy: Phage therapy can be combined with traditional antibiotics or other antimicrobial agents, potentially enhancing their effectiveness. By using phages alongside antibiotics, the therapy can reduce the required dosage, limiting the selective pressure that drives the development of resistance.

Biofilm Eradication: Biofilms are complex bacterial communities that are highly resistant to antibiotics. Phages have demonstrated the ability to penetrate and disrupt biofilms, making them a potential solution for treating chronic and biofilm-associated infections. As told earlier, Antibiotics were highly effective in treating bacterial infections, leading to complacency and a diminished focus on alternative treatment approaches like phage therapy.

Unfortunately, at the same time, the widespread use or, better to say, abuse and misuse of antibiotics immensely contributed to the emergence of antibiotic-resistant bacteria, which later renewed interest in phage therapy. Ongoing research, clinical trials, and advancements in understanding phage biology, production, and delivery mechanisms are reviving phage therapy as a promising approach to combat bacterial infections. It is increasingly recognized as a potential solution to address the challenges posed by antibiotic-resistant bacteria, leading to renewed interest and investment in phage therapy worldwide.

That brings us to the question, what is modern phage therapy?

Modern Phage Therapy: Modern approaches to phage therapy have evolved to overcome some of the challenges and limitations of traditional methods[11] (Fig. 1). Here are some of the key modern approaches to phage therapy:

Phage Cocktails: Instead of using a single phage, phage therapy now often involves using phage cocktails. A phage cocktail consists of a mixed population of phages that can target different strains or species of infection-causing bacteria. Phage cocktails increase the likelihood of effectively targeting a broader range of bacteria and developing a protocol for feasible and effective therapeutics.

Phage Libraries and Screening: Phage libraries are collections of diverse phages that can be screened to identify the most effective against specific bacterial strains. By screening large libraries, researchers can identify phages with a high affinity for the target bacteria, increasing the chances of successful treatment.

Pharmacokinetics and Delivery Optimization: Understanding the pharmacokinetics of phages is crucial for their successful application. Research focuses on optimizing phage delivery methods, such as encapsulating phages in liposomes or hydrogels, to improve their stability, prolong their circulation time, and enhance their ability to reach the site of infection.

Combination Therapies: Phage therapy can be combined with other antimicrobial agents, such as antibiotics or antimicrobial peptides, to enhance treatment outcomes. Combining phages with antibiotics can reduce the required antibiotic dosage and minimize the development of resistance.

Against the Formation of Biofilms and Chronic Infections: Biofilms, complex bacterial communities surrounded by a protective matrix, are highly resistant to antibiotics. Phage therapy is being explored as a strategy to target and disrupt biofilms, making it a potential solution for chronic and biofilm-associated infections.

Preclinical and Clinical Trials: To establish the safety and efficacy of phage therapy, rigorous preclinical and clinical trials are being conducted. These trials help generate robust evidence for the effectiveness of phage therapy against specific bacterial infections and provide important data on optimal dosing, routes of administration, and patient selection criteria.

Regulatory Advancements: Regulatory agencies are increasingly recognizing the importance of phage therapy and working towards establishing clear guidelines and regulations for its safe and effective use. These advancements facilitate the integration of phage therapy into mainstream healthcare practices.

Phage Engineering and Modification: Genetic engineering techniques can be employed to modify phages to enhance their effectiveness. For example, phages can be engineered to express additional antimicrobial factors or to target specific bacterial virulence factors, making them more potent against antibiotic-resistant or pathogenic strains[12].

Recent clinical trials resoundingly demonstrate the transformative potential of phage therapy as a formidable line of defense against antibiotic-resistant infections. These studies underscore phage therapy's targeted adaptability, presenting a compelling vision of its role in modern medicine. Here are some exemplary cases from global research, each highlighting phage therapy's promise:

1. PhagoBurn (Europe): In this pioneering multicentre study, researchers tackled severe burn wound infections primarily caused by *Escherichia coli* and *Pseudomonas aeruginosa*. Despite encountering obstacles around phage stability, PhagoBurn illuminated critical aspects of phage formulation and administration, setting a precedent for future phage trials[13].
2. Cystic Fibrosis and Phage Therapy (USA): Clinical trials under the aegis of the Cystic Fibrosis Foundation focused on chronic lung infections by *Pseudomonas aeruginosa*, a resilient pathogen in cystic fibrosis patients. Compassionate use cases revealed considerable reductions in bacterial load and respiratory improvement, particularly among patients who had exhausted all conventional treatments[14].

3. Intra-Abdominal Infections (Yale University): Targeting the multi-drug resistant *Acinetobacter baumannii*, this Yale-led trial showcased phage cocktails' effectiveness in reducing infection markers, indicating phages' potential in complex, patient-specific treatments[15].

4. Mycobacterium abscessus Infections (University of Pittsburgh): Compassionate use of phages against the notoriously treatment-resistant *Mycobacterium abscessus* yielded profound results, including total bacterial eradication in some cases. This study shines as a beacon of hope for phage-based approaches in treating intractable infections[16].

5. Bone and Joint Infections (PHAGEinLYON, France): In this trial for intransigent bone infections caused by *Pseudomonas aeruginosa*, phage therapy, combined with antibiotics, demonstrated efficacy against biofilm-forming bacteria that often evade conventional treatments[17].

6. Diabetic Foot Ulcer Infections (Belgium): Chronic, drug-resistant infections in diabetic foot ulcers proved responsive to phage therapy, which significantly reduced bacterial levels and supported wound healing in this vulnerable patient population[18].

7. Staphylococcus aureus Bacteremia (Baylor College of Medicine): Focused on *Staphylococcus aureus* bacteraemia resistant to traditional antibiotics, phage therapy markedly reduced bacterial counts and decreased recurrence rates, illustrating its potential to revolutionize treatments for resistant bloodstream infections[19].

8. Endocarditis (Switzerland): Researchers targeted endocarditis, a severe infection of the heart lining, using phages against multi-drug resistant bacteria. Results suggested phages as a viable alternative for this life-threatening condition[20].

9. Chronic Wound Infections by Klebsiella pneumoniae (Georgia): A Georgian study on chronic wounds infected with multi-drug-resistant *Klebsiella pneumoniae* showcased significant bacterial reductions and enhanced healing when phages were used in conjunction with antibiotics[21].

10. Pseudomonas Bone Infections (UK): By disrupting the biofilms associated with bone infections, phage therapy allowed for better antibiotic penetration and infection resolution[22].

11. MRSA Wound Infections (Israel): Trials targeting methicillin-resistant *Staphylococcus aureus* (MRSA) exhibited phages' effectiveness in diminishing bacterial presence and facilitating wound healing, illustrating their potential against this formidable pathogen[23].

India's Advances in Phage Therapy

In India, a slew of recent trials demonstrates the potential for phage therapy in treating antibiotic-resistant infections, mainly where conventional treatments fall short:

1. Diabetic Foot Ulcer Treatment (ICMR): Aiming at antibiotic-resistant bacteria in diabetic foot ulcers, this ICMR-affiliated trial saw marked healing improvements and infection control, heralding new options for chronic infection treatment[24].

2. Chronic Osteomyelitis (CMC, Vellore): Phage therapy offered hope for intractable bone infections, reducing bacterial loads where antibiotics had previously failed[25].

3. Klebsiella Pneumoniae in ICUs (AIIMS): Targeting drug-resistant *Klebsiella pneumoniae* infections in intensive care patients, this study revealed a significant reduction in bacterial presence and improved patient outcomes[26].

4. Burn Wound Infections (Kasturba Medical College): Focused on phage therapy for burn patients with *Pseudomonas aeruginosa* infections, this trial observed faster healing and reduced antibiotic dependence[27].

5. UTI Treatments (NICED): For UTIs caused by resistant strains, phage therapy reduced symptoms and achieved quicker bacterial clearance, especially in multi-drug-resistant cases[28].

6. Staphylococcus aureus Respiratory Infections (PGIMER): Phage therapy significantly lowered bacterial loads in MRSA-related respiratory infections, suggesting new paths for resistant lung infection treatments[29].

7. Chronic Wound Healing (BHU): Under the guidance of Dr. Gopal Nath, studies at Banaras Hindu University demonstrated enhanced healing for diabetic foot ulcers and other chronic wounds, proving phage therapy's efficacy in infection control[30].

8. PhageLytix Initiatives: India's first phage therapy firm, PhageLytix, is advancing the field through custom phage cocktails for chronic infections, achieving high success rates in treating multi-drug-resistant infections[31].

9. Vitalis Phage Collaborations (Delhi): With local hospital partnerships, Vitalis Phage has implemented phage therapy in treating persistent infections like UTIs, showcasing practical, innovative applications outside of formal trials[32].

These studies illustrate phage therapy's vast potential as a revolutionary complement - or alternative - to antibiotics, signalling an era where personalized phage solutions could offer targeted, effective treatments against the tide of antibiotic resistance.

In the current review, we have focused on two things. The first one is on the aspect of Phage engineering. Our study looks at the decision of phage excision, which is controlled by the regulation of lytic and lysogenic cycles.

And the second one is how phage therapy would be designed against enteric bacteria. Enterobacteria are a group of bacteria residing in humans and animals' gastrointestinal (GI) tracts.

Enterobacteria

They are crucial to human health and have extremely critical medical significance because they are part of the normal gut microbiota, a complex community of microorganisms inhabiting the GI tract. In a symbiotic manner, enterobacteria contribute to several physiological functions, such as digestion, nutrient absorption, and vitamin synthesis. A diverse and balanced gut microbiota is essential for maintaining overall health. Some enteric bacteria are responsible for causing gastrointestinal infections in humans, like *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, and *Campylobacter*. These infections can lead to symptoms like diarrhoea, abdominal pain, fever, and, in severe cases, dehydration and organ damage[33]. Proper identification and treatment of enteric bacterial infections are essential for preventing complications and reducing the spread of disease. Also, specific *E. coli* and *Salmonella* strains have shown increasing resistance to commonly used antibiotics. Monitoring and understanding the antibiotic resistance patterns of enteric bacteria are crucial for implementing appropriate treatment strategies and preventing the spread of resistant strains.

Enteric Phages

Enteric phages, also known as faecal phages or coliphages, are a group of bacteriophages that specifically infect bacteria of the Enterobacteriaceae family (Fig. 2). The Enterobacteriaceae family includes various gram-negative bacteria commonly found in the gastrointestinal tract, such as *Escherichia coli* (*E. coli*) and *Salmonella* sp.

Enteric phages are naturally present in the environment, including sewage, rivers, and soil, and they can be isolated from human and animal faeces. These phages have been extensively studied for their potential applications in phage therapy and as indicators of faecal contamination in water sources[34].

The presence of enteric phages in water can indicate faecal pollution, as their abundance correlates with the levels of faecal bacteria. Monitoring and quantifying enteric phages can provide valuable information about the microbial quality of water and the potential risk of waterborne diseases.

In phage therapy, enteric phages have been investigated for their ability to target and kill specific strains of enteric bacteria, including antibiotic-resistant strains[35]. By infecting and lysing the target bacteria, enteric phages can effectively treat gastrointestinal infections caused by these pathogens[36]. However, it is essential to note that using enteric phages in phage therapy requires careful consideration and specific evaluation. Factors such as phage host range, safety, efficacy, and potential interactions with the host immune system must be thoroughly assessed before their application in clinical settings.

Enteric phages play a significant role in environmental and medical contexts, offering potential benefits in water quality monitoring and developing targeted therapies against enteric bacterial infections.

Modes of Phage Replication

The lytic and lysogenic life cycles (Fig. 3) are two primary modes of replication and propagation observed in enteric bacteriophages. Two sets of genes control the life cycle of most bacteriophages; one corresponds to the lytic cycle of the phage, while the other codes for the lysogenic ones. The developmental or transcriptional switch that determines which pathway dominates will be extremely critical for determining the fate of the phage[37].

The bacteriophages replicate immediately after injecting their genetic material into their host cell, producing progeny phages ready for a new infection. The number of newborn phage particles increases gradually until it reaches a limit where the host bacteria can no

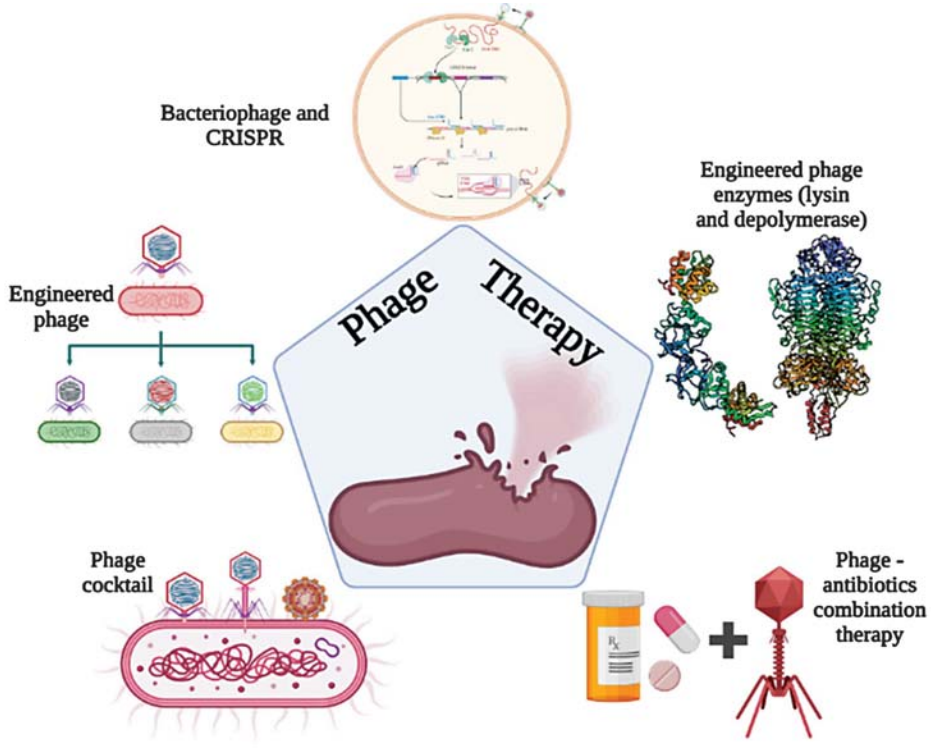

Fig. 1. Bacteriophage Therapy.

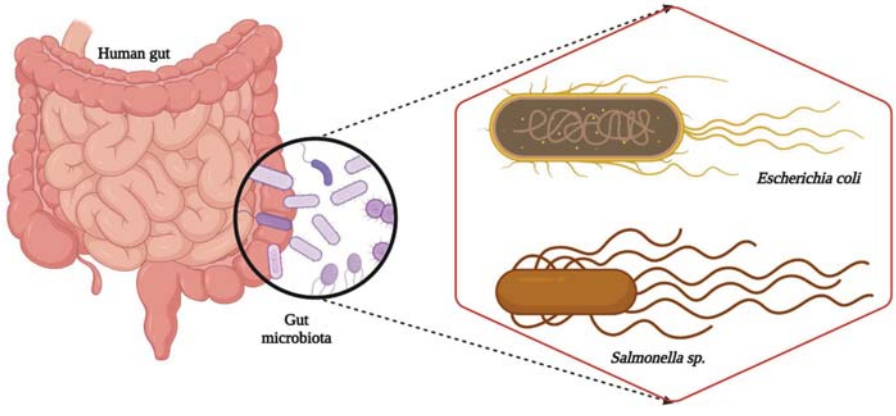

Fig. 2. Gut bacteria in humans.

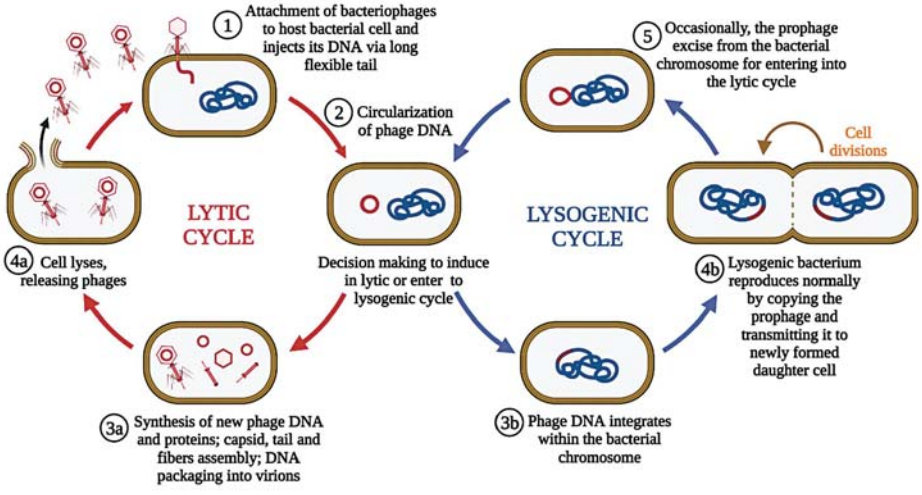

Fig. 3. Life cycle of bacteriophage.

longer host the newly produced phages. This breaks the bacteria's outer membrane by the phage-encoded lysis machinery and releases the bacteriophages.

The early lytic genes are switched on in this life mode, enabling DNA replication and immediate transcription after infection. Two proteins derived from A and B genes are essential for DNA replication. The first initiates the replication by the modified rolling circle mechanism[38, 39, 40], and the second protein works as a helicase loader[41]. To turn on the late functions, a third protein called the transcriptional activator Ogr must interact with the RNA polymerase, activating the transcription of the structural genes and the genes needed to lyse the host cell[42].

In the lysogenic life mode, the phage inserts its genetic material into the bacterial host chromosome by site-specific recombination between the phage attachment site *att*P and the host attachment site *att*B.

The integrase protein controls the interaction process between *att*P and *att*B, whereas the host-encoded protein called integration host factor (IHF) plays the structure-related responsibilities[43,44]. The junctions between the prophage and the bacterial DNA are *att*R and *att*L. After the integration event, the newly formed prophage performs replicates whenever the host replicates. The process continues until the phage is released from the host genome and enters the lytic cycle. The separation of host and phage DNA is called the excision process. It required the involvement of proteins like excisionase or Cox from phage in addition to the integrase and IHF). Here are the salient features of each cycle:

Lytic Life Cycle

In the lytic life cycle, the process begins with the phage attaching to specific receptors on the surface of the bacterial host, ensuring precise recognition of its target. Once bound, the phage injects its genetic material, either DNA or RNA, into the host cell's cytoplasm, while the protein coat (capsid) remains outside. Inside the host, the phage hijacks the bacterial machinery, overriding the normal cellular processes and redirecting them to replicate viral DNA and synthesize phage-specific proteins necessary for building new virions. As the host's resources are fully exploited, the newly formed genetic material and proteins are assembled into complete phage particles, each capable of infecting new bacterial cells. When assembly is complete, the bacterial cell undergoes lysis, rupturing and releasing a large number of progenies phages into the surrounding environment. These released phages are free to infect other susceptible bacterial cells, initiating additional lytic cycles. This life cycle is characterized by its efficiency and speed, leading to the rapid multiplication of phages and the ultimate destruction of the host cell. It underscores its potential for therapeutic applications where quick bacterial elimination is required.

Lysogenic Life Cycle

In the lysogenic life cycle, the phage begins by attaching to specific receptors on the surface of the bacterial cell, ensuring precise recognition, much like in the lytic cycle. Once bound, the phage injects its genetic material (DNA or RNA) into the bacterial cytoplasm. However, instead of initiating replication and assembly, the phage DNA integrates into the bacterial chromosome, becoming a prophage. This integration is mediated by specialized proteins and enzymes encoded by the phage, allowing the viral DNA to merge seamlessly with the host's genome. The prophage remains dormant in this state, becoming a stable part of the host's genetic material.

As the bacterium grows and divides, it replicates both its own DNA and the integrated prophage DNA, passing it on to daughter cells during reproduction. Throughout this phase of lysogeny, the prophage coexists peacefully with the host, causing no immediate harm or noticeable viral activity. The absence of overt replication ensures the phage can remain hidden within the host's genome for extended periods, effectively preserving both the bacterium and the prophage. This dormant state offers the phage a survival strategy, enabling it to persist across generations until certain environmental triggers prompt a switch to the lytic cycle, initiating active replication and host cell destruction.

Induction

Under specific conditions, such as exposure to environmental stressors or other triggering factors, a prophage - the dormant form of a bacteriophage integrated into the bacterial genome - can be induced to transition from the lysogenic state to the lytic cycle. This induction process typically occurs in response to signals such as UV radiation, nutrient deprivation, oxidative stress, or the presence of antibiotics, which can compromise the stability of the lysogenic relationship. During induction, the prophage excises itself from the host genome through a precise genetic recombination process, reactivating its viral program. Once excised, the phage proceeds through the steps of the lytic cycle, leading to the production of new viral particles, ultimately causing the lysis of the host cell and the release of progeny phages into the environment.

The lysogenic life cycle, on the other hand, allows the phage to integrate its DNA into the host bacterium's genome, where it can remain dormant for prolonged periods without initiating cell lysis. This strategy provides a survival advantage, enabling the phage to persist through adverse environmental conditions by essentially "hitching a ride" within a stable bacterial host. During this latent phase, the prophage may also play a crucial role in bacterial evolution. It can facilitate horizontal gene transfer, passing along genes that may enhance bacterial fitness, such as antibiotic resistance or virulence factors. This genetic exchange can have significant implications for bacterial pathogenicity and adaptation.

The regulation of the switch between lysogeny and the lytic cycle is a complex and tightly controlled process influenced by several factors. These include the availability of host-specific regulatory proteins, such as the phage repressor proteins, which maintain the prophage in a dormant state by inhibiting the transcription of lytic genes.

Environmental cues, such as shifts in temperature, pH, or the presence of certain chemicals, can also play a pivotal role in determining whether the phage remains lysogenic or transitions to the lytic phase. Additionally, the genetic makeup of both the phage and the host bacterium affects this decision, as certain prophages are more prone to induction under specific conditions[45].

In the current study, we aim to understand some of the DNA-binding proteins which play critical tole in governing the transition between the lytic and lysogenic phases in bacteriophages. These proteins act as molecular switches, determining whether a phage enters the lytic cycle - leading to the destruction of the host bacterium - or adopts the lysogenic cycle, where the viral genome integrates into the host's DNA and remains dormant until triggered. Understanding how these proteins regulate the switch between these two pathways is crucial, as it directly impacts the development of engineered phages for therapeutic applications.

Unfortunately, very little information about those family of proteins are available in the literature making it difficult to select them for further studies. In this review we have collected basic information about some of the representatives (origin, functional details, structural information etc.) especially belonging to enteric phages. Our focus will be on key DNA-binding proteins, including Cox, Cro, C, Integrase, and Excisionase, which play pivotal roles in influencing the outcome of the lytic-lysogenic decision. These proteins coordinate a cascade of molecular events, such as gene regulation, viral integration, and excision from the bacterial genome, that determine whether the phage remains latent or initiates bacterial lysis. Each of these proteins offers unique insights into phage biology: Cro promotes the lytic cycle by repressing lysogenic genes, while C facilitates lysogeny by maintaining the prophage state. Integrase and Excisionase are involved in the precise insertion and excision of the viral genome, further shaping the phage's replication strategy.

Investigating the in-detailed mechanisms of these proteins, their regular mechanistic details will certainly help advancing the field of engineered phage therapeutics. A deeper understanding of these molecular switches could allow scientists to manipulate phages more effectively - either by reinforcing their lytic potential to maximize bacterial clearance or by delaying lysis for strategic intervention. Moreover, targeted modulation of these proteins could enable the design of phage therapies tailored to specific bacterial infections, offering a precise, adaptable solution to combat multidrug-resistant bacteria[46].

We sincerely hope that the current review will lay the foundation for innovative phage engineering strategies, where fine-tuning the lytic-lysogenic balance through protein manipulation is critical to developing robust, next-generation therapeutics. The outcome of those biochemical mechanistic studies could significantly enhance the reliability and efficacy of phage therapy, pushing the boundaries of antimicrobial strategies and providing a powerful tool to outpace antibiotic-resistant pathogens[47].

Cox Protein

The name Cox is actually an abbreviation of "Control of x" (Cox) which is provided because of the enzyme's involvement in controlling and regulating the decision between the lytic and lysogenic cycles of the phage. Cox protein is primarily present in Enterobacterial phages. It is found mostly in P2 and P2-like bacteriophages (Enterobacteria phage HK239, Enterobacteria phage 18, Enterobacteria phage HK239, Enterobacteria phage PhiD252 and *Salmonella* phage SEN5 and SEN4, etc.)[44].

Cox is a DNA-binding, multi-functional phage protein that acts as a transcriptional regulator. The function of Cox involved influencing the expression of key genes, which determines the fate of the infected bacterial cell. Its primary function is to repress the expression of genes involved in the lytic cycle and promote the lysogenic cycle, allowing the phage to integrate its genetic material into the host bacterium's genome and establish a lysogenic state[48].

During the lysogenic cycle, the Cox protein binds to specific DNA sequences, known as operator sites, within the phage genome. This binding prevents gene expression in the lytic cycle, including those responsible for viral replication and lysis of the host cell. By repressing these genes, the Cox protein helps maintain the prophage within the bacterial genome, allowing the phage to coexist with the host bacterium[48].

Various factors, such as environmental conditions and the presence of certain stressors, influence the decision to enter the lytic or lysogenic cycle. Interestingly Cox protein also plays a critical role; in response to specific triggers, the Cox protein can be inactivated or it's binding to operator sites disrupted, allowing the expression of lytic genes and initiation of the lytic cycle. This results in the production of new phage particles and lysis of the infected bacterium.

Cox protein is a small one, generally consisting of 91 amino acids (Fig. 4). No prior literature confidently determines the oligomeric state of this protein, but the hypothesis predicted a multi-oligomeric state while binding to DNA.

X-ray crystallography (X-Ray) was used to determine the structural features of the Cox protein; only one structure exists in the literature (PDB ID: 4LHF)[49]. Like many other lytic-lysogenic regulator proteins, the Cox protein's structural features include mostly DNA binding and oligomerization domains and uses an HTH super secondary structure for DNA binding. But it should always be remembered that the Cox protein and its regulatory role in the lytic-lysogenic decision are not universal across all bacteriophages. It is specific to certain temperate phages, which can choose between the lytic and lysogenic cycles[43].

The Cox protein and other such regulatory proteins and the mechanisms contributed to the precise control of phage life cycle dynamics are specific according to their presence in a bacteriophage[50]. Different phages may have distinct regulatory systems and regulatory proteins involved in controlling their life cycles.

Cro Protein

Cro protein, alternatively termed "Control of Repressor Operator protein" (CRO), is also a regulatory protein like Cox, found in certain enteric bacteriophages[51]. It is critical in controlling the decision between these phages' lytic and lysogenic cycles[52]. The Cro protein, like Cox, also acts as a transcriptional regulator and regulates gene expression within the phage genome. It interacts with specific DNA sequences called operator sites and influences gene expression in the lytic and lysogenic cycles.

In the lysogenic cycle, the Cro protein can repress the expression of lytic genes by binding to operator sites within the phage genome. This repression prevents the production of phage particles and lysis of the host bacterium, allowing the phage to establish a lysogenic state. The Cro protein helps maintain the prophage within the bacterial genome. During the lytic cycle, the Cro protein is typically inactivated or displaced from operator sites, allowing the expression of lytic genes. This leads to the production of phage particles, the lysis of the infected bacterium, and the release of newly formed phages. The regulation of the lytic-lysogenic decision is a complex process involving various regulatory proteins and mechanisms. The Cro protein is one of the regulatory proteins involved in this decision, specifically in controlling gene expression within enteric bacteriophages.

Experimental techniques such as X-ray crystallography (X-Ray) and nuclear magnetic resonance (NMR) spectroscopy were used to determine the structural features of the Cro protein (Fig. 5).

Ten structures are solved, and their PDB IDs are the following: 1COP (NMR)[53]; 1D1L (X-RAY); 1D1M (X-RAY); 1ORC (X-RAY); 2A63 (NMR); 2ECS (X-RAY); 2ORC (NMR); 2OVG (X-RAY); 3ORC (X-RAY); 4CRO (X-RAY)[54]; 5CRO (X-RAY)[55]; 6CRO (X-RAY). The structural features of the Cro protein can vary depending on the specific enteric bacteriophage and its evolutionary lineage. However, mostly three features are significant, a) DNA-binding domains, b) Macromolecular interaction domain and c) flexible regions[56].

DNA binding Domain: The Cro protein typically contains one or more DNA-binding domains that allow it to interact with specific DNA sequences known as operator sites. These domains enable the protein to recognize and bind to specific regions of the phage genome, thereby regulating gene expression.

Protein-protein interaction domains: Regulatory proteins like Cro often possess domains that facilitate interactions with other proteins that regulate the phage life cycle. These protein-protein interaction domains allow Cro to interact with other regulatory proteins, transcription factors, or co-factors, influencing the overall regulatory network.

Flexible regions: Some regulatory proteins, including Cro, may contain flexible regions that allow for

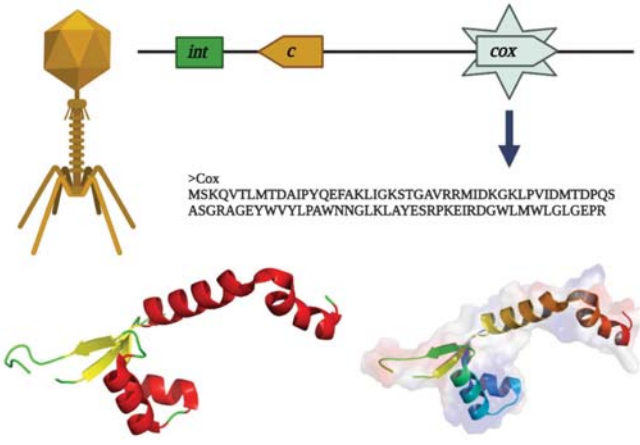

Fig. 4. Salient feature of Cox proteins in terms of sequence, structure and regulation.

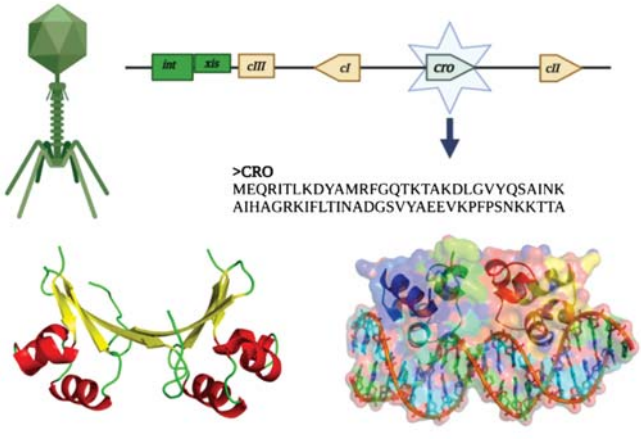

Fig. 5. Salient features of Cro proteins in terms of sequence, structure and regulation.

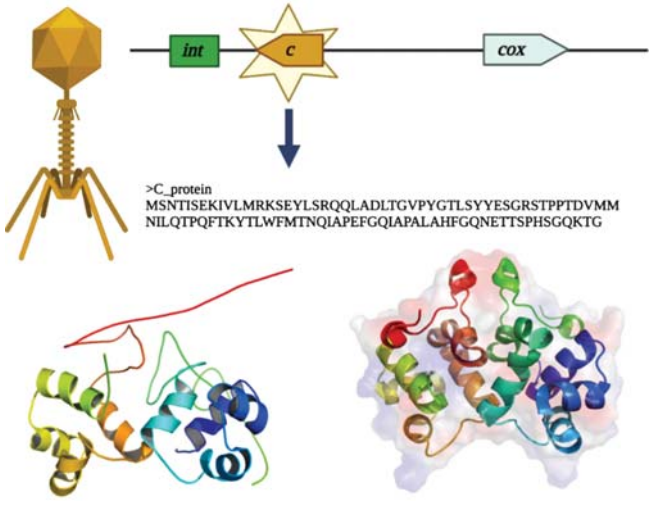

Fig. 6. Salient features of Cox proteins in terms of sequence, structure and regulation.

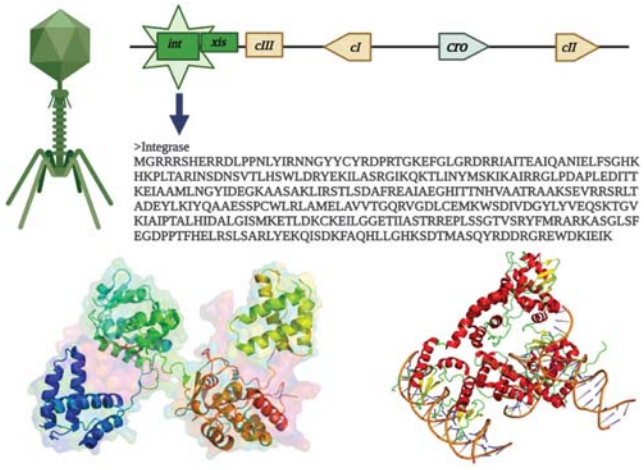

Fig. 7. Salient features of Integrase proteins in terms of sequence, structure and regulation.

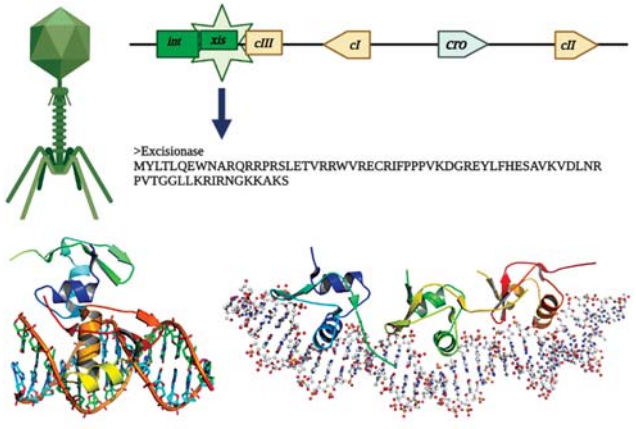

Fig. 8. Salient features of Excisionase proteins in terms of sequence, structure and regulation.

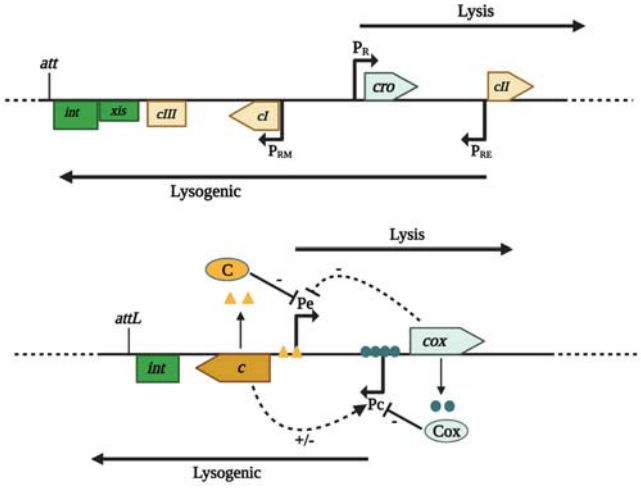

Fig. 9. Regulation of the Lytic-lysogenic cycle by different DNA-binding proteins.

conformational changes and dynamic interactions with DNA and other proteins. These flexible regions can play a role in modulating the binding affinity and specificity of the protein to its target DNA sequences.

Interestingly, the overall structure of the Cro protein can vary among different bacteriophages and even strains within the same phage species. This variation can include differences in the arrangement of domains, the presence of additional functional motifs, or the overall size of the protein.

C Protein

The protein "C", which is mainly found in P2 enteric phages, is a critical regulator involved in the lytic-lysogenic decision-making process of the P2 bacteriophage. Like other repressor proteins, Cox, CRO, and C are small proteins consisting of 99 amino acids. One structure of Protein C is determined using macromolecular crystallography (PDB ID: 2L49)[57]. The repressor protein shows structural similarity to other DNA-binding proteins. Analysis of the structure indicates the presence of two repressor-binding sites with a typical DNA binding Helix-Turn-Helix (HTH) super secondary structure (Fig. 6).

C Protein has some characteristic features making it distinct from the other regulatory proteins. They are the following:

Dual Function: C Protein has a dual function and can act as a transcriptional repressor and an anti-repressor. Its primary role is to control the switch between the lytic and lysogenic life cycles of the P2 phage[58].

Repressor Activity: Protein C binds to specific DNA sequences called operator sites within the P2 phage genome as a transcriptional repressor. This binding prevents the expression of genes required for the lytic life cycle, thereby repressing the production of lytic proteins and halting the lytic cycle.

Anti-repressor Activity: In addition to its repressor function, protein C can also act as an anti-repressor. Under certain conditions, protein C interacts with a repressor protein called CI, produced by the P2 phage. This interaction results in the inactivation of CI, releasing the repression on the lytic genes and promoting the switch to the lytic life cycle.

Decision-Making Process: Protein C is a critical player in the decision-making process between the lytic and lysogenic pathways. Various environmental cues and signals influence its levels and activities, such as DNA damage or stress conditions, which can trigger the switch from lysogeny to the lytic cycle.

Phage Fitness: Protein C's role in the decision-making process ensures the survival and fitness of the P2 phage. By regulating the switch between the lytic and lysogenic life cycles, the phage can adapt its strategy based on environmental conditions and optimize its successful replication and dissemination chances. In summary, the P2 phage protein C plays a pivotal role in the lytic-lysogenic decision of the P2 bacteriophage. Its dual function as a repressor and anti-repressor allows for dynamic control of the switch, enabling the phage to respond to environmental cues and determine the most advantageous life cycle for its survival and propagation.

Integrase Protein

Two phage-encoded proteins mediate site-specific recombination in bacteriophage, and integrase is one of them. The protein is a crucial component involved in the lytic-lysogenic decision-making process of bacteriophages. DNA sequence analysis predicts a molecular weight of 40.33KD, comprising 356 amino acids. Among them, 69 amino acid residues are essential, and 46 are acidic. 35% of the first 20 amino acids are crucial to the amino-terminal portion of integrase.

The structures of the protein are solved using macromolecular crystallography (PDB IDs: 1AE9[59]; 1KJK[60]; 1P7D[61]; 1Z19; 1Z1B; 1Z1G[62]; 2OXO[63]; 2WCC[64]; 5J0N[65]. Integrases from different bacteriophages can exhibit structural homology, particularly within their catalytic domains.

Despite variations in specific amino acid sequences and loop regions, they may share similar overall fold and secondary structure elements. Some key structural characteristics include; a) The catalytic domain is responsible for the protein's enzymatic activity. For doing this, it contains the essential residues involved in DNA binding, cleavage, and recombination. It plays a central role in mediating the site-specific recombination process required for the integration and excision of the phage genome. b) Integrases have DNA binding regions that enable specific recognition and binding to the attachment sites on both the phage genome and the host bacterial chromosome. c)Though not all, most integrases can form oligomers, contributing to their functionality. This domain allows integrases to interact with multiple binding sites on the phage genome and the host chromosome, facilitating recombination. d) besides these essential features, Integrases often contain protein-protein interaction domains

that enable their interactions with other phage-encoded or host regulatory proteins. These interactions can modulate the integrase activity and contribute to regulating the lytic-lysogenic decision e) Some integrases possess flexible regions or linkers that connect different domains within the protein. These flexible regions allow for conformational changes and structural flexibility, important for the enzymatic activity and interactions of integrases with DNA and other proteins (Fig. 7).

Here are the salient features of integrase and its role in this decision:

Enzymatic Activity: Protein integrase possesses enzymatic activity, specifically DNA recombinase activity. This activity allows integrase to catalyze the site-specific recombination process, which enables the integration and excision of the phage genome into/from the host bacterial chromosome.

Integration into Host Genome: Integrase is central in integrating the phage genome into the host bacterial chromosome during the lysogenic cycle. It recognizes and binds to specific DNA sequences known as attachment sites or attP sites on the phage genome and the bacterial chromosome. Integrase then catalyzes the recombination between these sites, resulting in the stable integration of the phage DNA into the host genome.

Excision from Host Genome: In the event of the switch to the lytic cycle, integrase is responsible for the excision of the phage genome from the host chromosome. This process involves the recognition and recombination between specific DNA sequences called attachment sites or *att*B sites on the phage genome and the bacterial chromosome, leading to the excision of the phage DNA.

Control of Lytic-Lysogenic Decision: The activity of integrase is tightly regulated and is influenced by various factors, including environmental cues and phage-encoded regulatory proteins. These factors dictate the lytic-lysogenic decision of the bacteriophage. Depending on the prevailing conditions, integrase either catalyzes the integration of the phage genome into the host chromosome, leading to lysogeny or promotes the excision of the phage DNA, triggering the lytic cycle.

Stability and Maintenance of Lysogeny: Once the phage genome is integrated into the host chromosome, integrase plays a role in maintaining the strength of the lysogenic state. It ensures that the phage genome is faithfully replicated and is stably inherited during host cell division.

Prophage Formation: The activity of integrase is essential for the formation and maintenance of a prophage, which refers to the integrated phage genome within the host chromosome during the lysogenic cycle. Integrases from different phages exhibit specificity for their respective attachment sites, ensuring site-specific recombination and integration.

Overall, protein integrase is a critical enzyme that regulates the lytic-lysogenic decision of bacteriophages. Its ability to catalyze site-specific recombination enables the integration and excision of the phage genome, thus determining whether the phage adopts a lysogenic or lytic lifestyle.

Excisionase Protein

The protein excisionase is a phage protein encoded by the Xis gene. It is involved in excisive recombination by regulating the concerned intasome's assembly, inhibiting viral integration. Excisionase is a protein that plays a significant role in the lytic-lysogenic decision-making process of bacteriophages (Fig. 8). The protein is a small one, comprising 72 amino acids. The protein structure is solved by the approach of protein crystallography and nuclear magnetic spectroscopy resulting in high-resolution systems (PDB IDs: 1LX8; 1RH6; 2IEF; 2OG0; 5J0N; 6P0S; 6P0T; 6P0U)[65].

Analysis of those structures reveals an unusual winged helix structure in which two alpha helices are packed against two extended strands. Also present in the structure is a two-stranded anti-parallel beta-sheet, whose strands are connected by a four-residue wing. But the breakthrough came through a relatively low-resolution structure (PDB ID: 5J0N) of a Holliday junction complex, revealing mechanisms governing a highly regulated DNA transaction explaining how excisionase cooperates with other proteins like integrase and Integration host factor (IHF).

From the structural understanding added with other biochemical, biophysical, molecular biology, and genetic studies, we have found some of the salient features of excisionase and its role in this decision: Regulatory Protein: Excisionase is a phage-encoded regulatory protein typically produced during a bacteriophage's lysogenic cycle. Its production is tightly regulated and often dependent on specific environmental cues or triggers. Activation of Excision: Excisionase is responsible for activating the excision process, which involves the removal of the phage genome from the host bacterial chromosome. It interacts with other regulatory proteins, such as integrase or

repressor, to initiate the excision reaction[66, 67]. Interaction with Integrase: Excisionase interacts with integrase, the enzyme responsible for site-specific recombination, to stimulate the excision of the phage genome. This interaction can modify the integrase activity, disrupting the integrated phage genome and the subsequent release of the phage DNA.

Temporal Control: The production of excisionase is often temporally regulated and coordinated with other regulatory proteins involved in the lytic-lysogenic decision. This control ensures that the excision of the phage genome occurs at the appropriate time, facilitating the transition from lysogeny to the lytic cycle.

Environmental Signals: The production or activation of excisionase can be influenced by specific environmental signals or stress conditions. These signals can trigger the switch from the lysogenic to the lytic cycle, producing excisionase and subsequent excision of the phage genome.

Role in the Lytic-lysogenic Decision: Excisionase plays a critical role in determining the fate of the phage and influencing the lytic-lysogenic decision. Its activation and subsequent excision of the phage genome promote the transition to the lytic cycle, producing viral progeny and host cell lysis. Thus, excisionase contributes to the decision-making process by shifting the phage from lysogeny to the lytic lifestyle.

We have talked about a few critical proteins (Cox, Cro, C, Integrase and Excisionase) playing key roles in deciding the phages' lytic and lysogenic life cycle. It is important to note that those proteins' specific features and mechanisms can vary among different enteric phages. The proteins' regulation and role in the lytic-lysogenic decision depend on the phage's genetic and regulatory factors and the interplay between excisionase and other proteins involved in the decision-making process. Also, it's important to note that while there are general patterns concerning the structural features of those regulatory proteins, the specific structural details may vary based on the phage and the specific experimental studies conducted on it. Further research and structural characterization of individual proteins are necessary to obtain detailed insights into their specific structural features and functional mechanisms (Fig. 9).

Discussion:

The accelerating threat of antimicrobial resistance (AMR) presents a formidable challenge to public health, driven by the ability of bacteria to withstand formerly effective antibiotic treatments. This growing crisis has necessitated exploring innovative solutions, with phage therapy emerging as a compelling strategy.

Phage therapy offers a promising alternative or complement to antibiotics, especially in the fight against antimicrobial resistance (AMR), which has become a critical global health concern. Unlike broad-spectrum antibiotics, bacteriophages (phages) specifically target certain bacterial strains, making them highly selective and reducing the likelihood of disrupting beneficial microbiota. This specificity minimizes collateral damage to the host's natural microbial community, a significant advantage over conventional antibiotics. Phage therapy is particularly valuable against multidrug-resistant bacteria, where traditional treatments have failed, offering a potential solution for otherwise untreatable infections. Furthermore, phages can evolve alongside bacteria, potentially outpacing bacterial resistance mechanisms through natural selection. This adaptability makes them a dynamic tool in combating changing pathogens.

However, several challenges must be addressed for the widespread adoption of phage therapy. One major hurdle is the narrow host range of phages, meaning a specific phage may only target a limited number of bacterial strains, requiring precise identification of the infectious agent before treatment. Developing phage libraries to cover diverse bacterial infections and ensuring the availability of appropriate phages in clinical settings is another logistical challenge. Additionally, the human immune system may neutralize phages before they can act, reducing their therapeutic effectiveness. Regulatory challenges also complicate progress, as phage therapy falls outside conventional drug approval frameworks, making standardization, safety assessments, and clinical trials more complex. Furthermore, bacterial resistance to phages can still occur, necessitating ongoing research into phage cocktails or engineered phages to maintain efficacy.

Despite these challenges, the importance of phage therapy in the current landscape of AMR cannot be overstated. With continued research and advancements in molecular biology, phage therapy has the potential to become a powerful tool in precision medicine, tailored to individual infections and integrated alongside antibiotics to create synergistic treatments. The development of phage cocktails, personalized phage therapies, and engineered phages is likely to play a critical role in overcoming the limitations and ensuring that phage therapy realizes its full potential in clinical applications.

Bacteriophages (phages), viruses that target bacteria, follow two distinct biological cycles - lytic and lysogenic

- each playing a pivotal role in bacterial control and phage engineering.

In the lytic cycle, the phage attaches to a bacterial cell and injects its genetic material. It hijacks the host's cellular machinery, directing it to replicate phage DNA and synthesize viral proteins. The phage components assemble into new viral particles, eventually causing the bacterial cell to rupture (lyse), releasing numerous progeny phages that infect other bacteria. This cycle is especially valuable for phage therapy, as the immediate destruction of bacterial cells helps in eradicating infections.

Conversely, the lysogenic cycle involves the integration of phage DNA into the host bacterium's genome, forming a prophage that remains dormant within the bacterial cell. The prophage replicates along with the host's DNA without causing harm. However, environmental triggers can activate the prophage, switching it to the lytic cycle. While lysogenic phages can provide long-term bacterial control, they are less suited for therapy because they risk transferring antibiotic resistance genes to other bacteria.

Phage engineering leverages molecular tools to enhance the therapeutic potential of phages. One focus is modifying phages to favour the lytic cycle, ensuring rapid bacterial elimination. Techniques like CRISPR-Cas and synthetic biology are employed to disable lysogenic genes, broadening the phage's host range and improving precision. Furthermore, engineered phages can be designed to deliver antibacterial enzymes or CRISPR payloads, targeting even multidrug-resistant bacteria with high specificity.

This study focuses on the roles of five pivotal DNA-binding proteins - Cox, Cro, C, Integrase, and Excisionase - that are instrumental in dictating the life cycle of enteric bacteriophages. These regulatory proteins determine whether a phage embarks on the lytic cycle, wherein it commandeers the host's cellular machinery to create new viral particles and ultimately lyses the cell, or enters the lysogenic pathway, integrating its genome with the host's DNA in a latent state until environmental cues prompt it to reactivate. This cycle-switching capability endows phages with remarkable adaptability, yet for therapeutic purposes, the lytic pathway is particularly valuable due to its immediate bacterial-killing effect.

Deciphering these proteins' intricate roles is crucial for advancing phage engineering, especially in crafting phages biased toward the lytic pathway to maximize bactericidal potency against multidrug-resistant strains. Understanding how Cox, Cro, C, Integrase, and Excisionase influence cycle selection at the molecular level allows for creating phages precisely engineered for clinical efficacy, providing a powerful new arsenal against drug-resistant infections.

Despite their vital roles, these proteins remain poorly documented, highlighting an urgent need for a dedicated repository encompassing DNA and protein sequences, evolutionary profiles, and detailed 3D structural and dynamic models. Such resources would be invaluable for tracking the conservation and variability of these proteins across bacterial species, revealing structural nuances that influence their regulatory roles. Evolutionary analyses could further clarify which protein elements are functionally essential, pointing to prime engineering targets for optimal therapeutic design.

Moreover, structural studies on protein-DNA interactions are essential, as they underpin the molecular decisions that toggle between the lytic and lysogenic states. Insights into how these proteins recognize specific DNA sequences and prompt structural shifts would clarify the precise mechanisms at play, transforming these academic findings into practical tools. With this knowledge, researchers could create phages that favor the lytic cycle exclusively, bypassing the lysogenic option where immediate bacterial destruction is necessary.

By unravelling the structural and functional landscapes of Cox, Cro, C, Integrase, and Excisionase, we set the stage for the next era of phage engineering. The ability to develop customized phages that selectively target specific bacterial pathogens represents a leap forward in phage therapy. As we deepen our grasp of these regulators, we unlock the potential to wield phages as precision instruments in the battle against antimicrobial resistance, positioning them as more than adjuncts to antibiotics - instead, as adaptable and independent therapies that can evolve alongside bacterial pathogens.

This research thus lays the groundwork for innovative, personalized approaches in phage therapy, creating new avenues for combating multidrug-resistant infections and safeguarding global health.

Our study paves the way for next-generation phage-based therapies by addressing these key knowledge gaps. The insights gained will enable precision medicine approaches that tailor phages to specific infections while also opening the door to synergistic therapies combining phages with antibiotics. As phage engineering advances, innovations such as personalized phage cocktails and engineered phages will become crucial in the fight against

AMR. Ultimately, this research serves as a framework for the future, propelling phage therapy to the forefront as a formidable tool against drug-resistant infections.

As antibacterial resistance (ABR) spirals into a global crisis, the need for visionary solutions has never been more pressing. Enter phage therapy - a strategy harnessing bacteriophages specialized viruses that exclusively target bacteria. Unlike the blunt impact of broad-spectrum antibiotics, phages strike with remarkable precision, attacking only specific bacterial strains and sparing the beneficial microbiota. This selective approach, coupled with their inherent ability to evolve alongside bacteria, offers phages a dynamic advantage in the relentless race against resistance, making them invaluable allies against multidrug-resistant infections.

Yet, the path to realizing the full potential of phage therapy is not without its challenges. Phages' narrow host range demands exact bacterial identification and an extensive library of phages to address varied infections. Moreover, regulatory frameworks remain ill-fitted to phage therapy, while the human immune response poses another obstacle by potentially neutralizing phages before they can act.

Nevertheless, the promise of phage therapy endures. Revolutionary strides in molecular and synthetic biology, particularly through CRISPR-Cas technologies, are paving the way to engineer phages for targeted, lytic activity - maximizing bacterial destruction while minimizing collateral effects. In the current study we have discussed the critical role of five regulatory proteins - Cox, Cro, C, Integrase, and Excisionase - that govern the phage's life cycle decision-making, a fundamental insight for advancing therapeutic phage design.

We are forging the path toward phage-based precision medicine by addressing key gaps in phage biology and leveraging advanced engineering. Through customized phage cocktails and synergistic use with antibiotics, phage therapy is poised to redefine the battle against ABR, reshaping the landscape of infectious disease treatment and fortifying global health for the future.

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Conflict of interest

There is no conflict of interest. ❐

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