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The Role of Temperate Bacteriophages in Bacterial Infection

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Abstract

Bacteriophages are viruses that infect bacteria. There are an estimated $10^{31}$ phage on the planet, making them the most abundant form of life. We are rapidly approaching the centenary of their identification, and yet still have only a limited understanding of their role in the ecology and evolution of bacterial populations. Temperate prophage carriage is often associated with increased bacterial virulence. The rise in use of technologies, such as genome sequencing and transcriptomics have highlighted more subtle ways in which prophages contribute to pathogenicity. This review discusses the current knowledge of the multifaceted effects that phage can exert on their hosts and how this may contribute to bacterial adaptation during infection.

Introduction: Lifestyle Choices: A good work-life balance

Bacteriophages (phage) are viruses that infect and replicate within bacterial hosts and are ubiquitous and abundant in every niche studied so far on the planet (Roux et al., 2015). They are broadly divided into two categories. Virulent phage follow a strictly productive lytic lifecycle whereas temperate phage switch between dormant and productive states. All phage infect the host bacterium by binding to specific surface receptors and injecting their genome into the cytoplasm. Virulent (lytic) phage infection immediately commandeers the bacterial replicative machinery for multiplication. Phage genes encode structural head and tail proteins and lytic enzymes that cause bacterial cell lyses, releasing lytic phage progeny into the environment. The characteristics of lytic phage offer an attractive alternative to antibiotics. Phage therapy has been widely used in the former Soviet Union (Hraiech et al., 2015) and
rapid spread of multi-drug-resistant infections has prompted renewed interest in phage-based therapies worldwide.

Temperate (lysogenic) phage follow an alternative life cycle involving integration of their genome into the host chromosome to become a prophage. In this state the phage DNA replicates along with the host cell (lysogen) and is maintained in the bacterial population. Lysogenic phage can switch to a lytic lifecycle, particularly in response to environmental stresses (Figure 1). Lambdoid phage employ repressor genes such as cl, which act as a genetic switch to control the balance between lysis and lysogeny (Ptashne, 2004). Expression of these repressors prevents the lytic pathway and maintains the prophage state. The CI repressor also inhibits integration of any incoming phage genomes conferring immunity to super-infection. There are a wide range of other phage-resistance mechanisms (reviewed in (Labrie et al., 2010).

The balance between lytic and lysogenic states is thought to be largely dependent on the metabolic condition of the bacterial host cell (Lieb, 1953). Temperate phage infection tends towards lysogeny in starving cells and this is thought to be a phage survival tactic during periods of resource limitation (Stewart & Levin, 1984). Integration into the chromosome is facilitated by integrase and transposase enzymes that can act at specific sites or randomly. This means that lysogenic phage can drive bacterial diversity by introducing mutations with each integration event. Active prophage retain the ability to switch to a lytic cycle of productive replication. This occurs spontaneously in a proportion of cells within a population of lysogenic bacteria. Induction of lambdoid phages into the lytic cycle has been well characterised and often linked to the SOS response triggered by DNA damage. Prophage induction is thought to be another survival strategy to aid phage escape from a host cell at risk of death (Refardt & Rainey, 2010). Potent inducers of DNA damage and phage induction
include physical and chemical mutagens such as UV, mitomycin C and reactive oxygen species (Aanaes et al., 2011). Several antibiotics have also been shown to trigger the lytic cycle, particularly those that target DNA replication (fluoroquinolones such as norfloxacin and ciprofloxacin) (Matsushiro et al., 1999, James et al., 2001; Fothergill et al., 2011; Meessen-Pinard et al., 2012; López et al., 2014).

During lysogeny, mutations commonly lead to the formation of a defective (cryptic) phage, locking the once mobile element into the host chromosome (Fischer-Fantuzzi and Calef., 1964; Bobay et al., 2014). The frequency of defective (domesticated) prophage may be grossly underestimated. They can be hard to identify as genome degradation often results in deletion of recognisable phage genes (Mizutani et al., 1999, Bobay et al., 2014).

Prophage contribution to infection

Lysogenic infection and subsequent expression by the host of phage encoded genes is termed lysogenic conversion, and can have profound effects on bacterial phenotype. Prophages often encode “morons” that are not directly involved in viral replication and can confer a benefit to their bacterial host. Such genes are independent transcriptional units of DNA that are expressed whilst the phage is in the prophage state (Juhala et al., 2000).

Morons can include genes that enhance the virulence of their bacterial host, either directly (e.g. phage-encoded toxins), or indirectly, by enhancing the ecological fitness of bacteria during infection (Hacker & Carniel, 2001). The role of temperate phage in disease situations is thus becoming increasingly recognised.

The recent growth in whole bacterial genome sequencing has revealed high numbers of integrated prophage (Hayashi et al., 2001, Winstanley et al., 2009, Wang et al., 2010, Matos et al., 2013). Pathogenic strains have been shown to carry a greater proportion of
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phage-related genes than non-pathogenic strains (Busby et al., 2013), many maintaining multiple prophages in the same chromosome (Hayashi et al., 2001, Winstanley et al., 2009).

For example, the majority of the genetic difference between avirulent and virulent strains of Escherichia coli is due to mobile genetic elements, notably phages (Hayashi et al., 2001, Ohnishi et al., 2002). Table 1 summarises some of the major phage-encoded bacterial virulence factors that have been identified.

Exotoxins: The concept of lysogenic conversion was first introduced in 1927 when it was demonstrated that a filterable agent (later identified as a bacteriophage) could convert previously non-toxigenic Streptococci into toxin producers (Frobisher & Brown, 1927). It wasn’t until the 1950s that phage transduction was shown to be responsible for toxigenic conversion of avirulent Corynebacterium diphtheriae to produce a potent exotoxin and become highly pathogenic to the animal host (Groman, 1953, Groman, 1955). Since then there have been numerous reports of phage-encoded exotoxins that enhance the virulence of their bacterial hosts, including Vibrio cholera, Staphylococcus aureus, Clostridium botulinum and E. coli (reviewed in (Casas & Maloy, 2011)). Shiga toxins (Stx), major virulence factors of Shigatoxigenic E. coli (STEC) are produced by a group of temperate Stx phages. The stx2 genes are located in the phage late gene region and are expressed when the prophage is triggered into the lytic cycle (Wagner et al., 2002).

Phage-encoded exotoxins are likely to contribute to bacterial fitness, but the exact mechanism remains unclear (for a review on the evolution of bacterial virulence, see (Levin & Svanborg Eden, 1990)). Phage-encoded exotoxins are well characterised as they often have a large impact on bacterial virulence. However, prophage can have more subtle effects on host phenotype, conferring a benefit to the host bacterium by enhancing colonisation or competitiveness in an animal host (Fortier & Sekulovic, 2013).
Adhesion and Invasion: One of the crucial first stages of bacterial infection is attachment to cells. Some phage-encoded shiga-toxins provide extra virulence by facilitating adherence of STEC to gut epithelial cells in a murine model of infection (Robinson et al., 2006). Several stx phages (e.g. 933W isolated from E. coli O157:H7) also possess a lom gene homologue that encodes an outer membrane protein necessary for adhesion to human epithelial cells (Vica Pacheco et al., 1997). The prophage-encoded PblA and PblB platelet binding proteins of Streptococcus mitis strain SF100 play an important role in the pathogenesis, causing endocarditis (Bensing et al., 2001) and homologs with similar functions have been identified in prophage of Enterococcus faecalis (Matos et al., 2013).

Bacterial type III secretion systems (TTSS) are associated with attachment and invasion by secreting effectors directly into target host cells. There are many examples of prophages that contribute to these systems in several intestinal pathogens. A cryptic prophage, CP-933C, has been reported to positively regulate a TTSS in E. coli (Flockhart et al., 2012). Deletion mutants of the cryptic phage displayed reduced colonisation and persistence in an ovine model, through a reduced ability to adhere to epithelial cells (Flockhart et al., 2012). The Salmonella typhimurium prophage-encoded SopE is an effector protein secreted via the TTSS into intestinal epithelial cells to promote invasion (Mirold et al., 1999). Likewise the CJIE1-like prophage, carried by some isolates of Campylobacter jejuni, confers increased adherence and invasion in vitro (Clark et al., 2012). This phage has also been shown to alter host protein expression in the presence of bile salts (Clark et al., 2012).

Contributions to fitness in vivo: Once bacteria have successfully colonised a host, they must reproduce and evade the host immune system. Biofilms are a key feature of many bacterial infections and can be described as complex microbial communities, protected by a
secreted matrix of exopolysaccharides, proteins and DNA. Biofilm-associated bacteria exhibit increased resistance to immune attack and antibiotic treatment. Both active and cryptic prophage have been suggested to play a role in biofilm development of several pathogens, including *S. pneumoniae* (Carrolo *et al.*, 2010), *Bacillus anthracis* (Schuch & Fischetti, 2009) and *E. coli* (Wang *et al.*, 2010). Homologs of the filamentous phage Pf4, are widespread in clinical *P. aeruginosa* isolates, and play a crucial role in several stages of biofilm maturation. In particular Pf4 switches to a super-infective form within mature biofilms, aiding dispersal. This has been associated with increased virulence in a mouse model of infection (Rice *et al.*, 2009).

Enhanced growth rate upon lysogenic conversion is a common phenomenon (Bondy-Denomy & Davidson, 2014). The prophage SMP increases both growth rate and resistance to lysozyme resulting in enhanced virulence of its *Streptococcus suis* host (SS2) in a zebrafish model of infection (Tang *et al.*, 2013). A reduced rate of growth has been observed when cryptic prophages are deleted from *E. coli* K12 compared to wild-type (Wang *et al.*, 2010).

Mutational studies of the Liverpool Epidemic Strain (LES) of *P. aeruginosa* (isolated from the lungs of patients with cystic fibrosis (CF)), revealed a significant association of prophage genes with competitiveness in a rat model of chronic lung infection (Winstanley *et al.*, 2009). Mutations in several prophage genes exhibited up to 1000 fold reduced ability to establish infection and modified the expression of multiple virulence genes, including key factors associated with chronic infection (Lemieux *et al.*, 2015). These studies suggest that temperate phage influence multiple stages of infection and alter the fitness of phage-carrying bacteria in the host environment.

**Immune modulation and antimicrobial resistance:** Some prophage confer bacterial traits that are capable of actively modulating the immune system. Shiga toxin, produced by *E.
coli Stx-phage, is capable of inhibiting the innate immune response of human enterocytes by inhibiting the PI3K/Akt/NF-B signalling pathway. This leads to a subsequent decrease in chemokines CCL20 and interleukin-8, which are linked with the innate immune response (Gobert et al., 2007). Several temperate phage of P. aeruginosa have been shown to convert non-mucoid strains to mucoidy, a phenotype characterised by the overproduction of the polysaccharide alginate (Miller & Renta Rubero, 1984). This phenotype provides bacteria with a physical protectant that helps them to be refractory to both the immune system (Cabral et al., 1987) and to antibiotic treatment (Hentzer et al., 2001).

Antimicrobial resistance (AMR) genes have been identified on phage isolated from water (Colomer-Lluch et al., 2011), activated sludge (Parsley et al., 2010), faecal samples (Quiros et al., 2014), and the lungs of individuals with CF (Fancelli et al., 2011). These genes can be transduced, changing the antimicrobial susceptibility profile of their host (Zhang & LeJeune, 2008, Mazaheri Nezhad Fard et al., 2011). An important example of this includes the transfer of the Staphylococcal cassette chromosome mec (SCCmec), a defining feature of Methicillin Resistance S. aureus (MRSA). This pathogenicity island can harbour several AMR determinants that are transferable by phage (Maslanova et al., 2013). Phage of bovine Salmonellae have been shown to transduce the bla\textsubscript{CMY-2} gene, encoding resistance to third-generation cephalosporins (Zhang & LeJeune, 2008) and the Staphylococcal phage, TEM123 (isolated from food), was shown to confer beta-lactam resistance via a metallo-\beta-lactamase gene (Lee and Park, 2015). In this way, phage have been described as “vehicles of the resistome” and metagenomic analysis of DNA from the respiratory tract of CF patients has revealed the presence of phage-associated AMR genes (Rolain et al., 2011). Modi et al. (2013) observed an increase in phage-associated AMR genes \textit{in vivo} following antibiotic treatment of mice. Interestingly, they detected enrichment of disparate mechanisms to resist
both the administered drug and unrelated antibiotics. Furthermore, the evolved phage were shown to transfer AMR to naïve cultures from mouse microbiota. These findings suggest that phages play an important role in driving the evolution and spread of resistance and should be considered in control measures.

Phage abundance in the human environment

A phenomenal diversity of phage has been described in the natural environment, in the region of 50 viral species per litre of sea water, and up to 1 million species in 1 kg of marine sediment (Rohwer & Thurber, 2009). Prophages have been identified in ~60% of sequenced bacterial genomes (Roux et al. 2015). The influence of bacteriophages on the life histories and evolution of their hosts in these environments is multi-faceted. In addition to the selective pressures of predation, horizontal transfer of important genes (e.g. those involved in stress response, chemotaxis and metabolic pathways) aid niche adaptation (Rohwer & Thurber, 2009). There is less known about the density of natural bacteriophage populations in vivo, and particularly during bacterial infections. Phage virions have been detected in human sputa and faeces by electron microscopy (Ojeniyi et al., 1991) and isolated using plaque assays (Furuse et al., 1983, Fothergill et al., 2011). These studies report E. coli phage (coliphage) titres of up to $10^5$ p.f.u. $g^{-1}$ human faeces (Dhillon et al., 1976) and an association has been identified between high coliphage densities ($>1 \times 10^5$ p.f.u. $g^{-1}$) and disease (Furuse et al., 1983). Others have observed a shift from predominantly temperate, to virulent phages associated with human diarrhoeal disease; a reflection of modified intestinal microflora.
Metagenomic studies have begun to describe the human virome and have indicated that phage far out-weigh eukaryotic viruses both in number and diversity (Willner et al., 2011, Reyes et al., 2012). Sequencing techniques are not dependent on plaque assays to detect phages and can thus enumerate total phage abundance without the need for a susceptible bacterial host. An estimated $10^8$–$10^9$ bacteriophage particles per gram of human faeces (Kim et al., 2011), and approximately $10^3$ virotypes (mainly temperate) have been identified (Breitbart et al., 2003). 236 and 175 viral species have been identified in the oral cavity and the respiratory tract respectively (Willner et al., 2009, Willner et al., 2011).

Temporal, spatial and inter-individual variation in virome diversity has been observed in the gastrointestinal tract (Kim et al., 2011), oral cavity (Pride et al., 2012) and respiratory tract (Willner et al., 2009). However, there is little known about the balance between active phage virion densities and prophages in vivo. The development of new bio-informatic tools, such as VirSorter (Roux et al., 2015) that can assemble viral genomes from metagenomic and single-cell amplified genome data, hold promise for the elucidation of this dynamic phage-host relationship in complex communities.

**Effects of antibiotic treatment:** It is well established that some antibiotics can trigger the switch between lysogenic and lytic phage lifecycles; particularly the fluoroquinolones, that affect DNA replication. Production of Clostridium difficile phages, isolated from human faeces, has been shown to increase by 4-5 logs in response to fluoroquinolone treatment (Meessen-Pinard et al., 2012). Ciprofloxacin has been demonstrated to trigger the V583 phage lytic cycle in E. faecalis. This antibiotic is routinely used in therapeutic regimes including in the management of P. aeruginosa infection in (CF) (Fothergill et al., 2011, Matos et al., 2013). This has been linked with both upregulation of phage-related genes (Cirz et al., 2006) and increased production of phage virions (Fothergill et al., 2011). Free P. aeruginosa phage have been detected at high levels in CF patient sputa,
most likely as a result of induction by antibiotics and oxidative stress (James et al., 2012, James et al., 2015). Norfloxacin is a well-known inducer of stx-phage from STEC, resulting in increased toxin production [Matsushiro et al., 1999]. Clinicians are therefore advised to avoid treatment of suspected STEC infection with fluoroquinolones (Nassar et al., 2013).

Long-term antibiotic treatment is likely to play a crucial role in the dynamics between prophage and their hosts in vivo. A longitudinal study of CF patient sputa tracked the density of six \textit{P. aeruginosa} phage that are all maintained as active prophages in the same LES chromosome. A consistently high density of DNA from LES phage virions (10$^{4}$ – 10$^{9}$ copies µl$^{-1}$) was observed that correlated positively with LES host numbers over a 2 year period. Free-phage density exceeded specific bacterial host density (11-90-fold), consistent with ongoing lytic activity. This was expected as CF patients are often treated with high doses of intravenous antibiotics during exacerbation of symptoms. Surprisingly, there was no correlation between LES phage density and treatment of exacerbated symptoms. These patients were subject to variable cocktails of different antibiotic classes over several years irrespective of exacerbations (James et al., 2015). Not all antibiotics induce the phage lytic cycle; in fact some are known to suppress lytic activity (Fothergill et al., 2011). As next generation sequencing technologies advance, the interaction between antibiotics, phage and their hosts during chronic infections can be teased apart in further longitudinal studies.

\textbf{Role of phage in bacterial adaptation}

It is no surprise that phage can be intimately involved in the adaptation and evolution of their bacterial hosts to drive bacterial diversification through numerous mechanisms. Lytic bacteriophages obligately kill their hosts placing a strong antagonistic selective pressure on
bacteria to avoid infection. The “kill the winner” hypothesis posits that the competition
specialists in a bacterial population become targets of bacteriophages. The subsequent
reduction in the “winners” selects for diversity in the population (Winter et al., 2010). The
obvious effects of lysogenic conversion of bacterial hosts have been well documented. The
carriage of additional genes during lysogeny can increase bacterial population diversity
through a less antagonistic selection pressure than lytic infection. However, the more subtle
effects of temperate phage on the adaptation of bacterial populations require further
exploration. Temperate bacteriophages can also drive host genome evolution through gene
disruption, duplication, transduction or by acting as anchor points for major chromosomal
rearrangements.

**Gene Disruption** frequently occurs through insertional inactivation. As an example
of negative lysogenic conversion, Staphylococcal phage L54a has been shown to integrate
into the lipase-encoding gene (*geh*) resulting in a loss of phenotype (Lee & Iandolo, 1986).
Another *S. aureus* phage, φ13, has integrated into the 5' end of the *hlb* gene, causing a loss of
beta-toxin expression (Coleman et al., 1991). *E. coli* phage Mu (mutator) was the first
identified example of a bacteriophage causing mutations in the host chromosome. Mu
lysogens were observed to display differences in their nutritional requirements through
phage-mediated disruption of gene function (Taylor, 1963). Phage Mu is transposable,
meaning it can integrate into random sites of the host chromosome (Bukhari & Zipser, 1972)
unlike many other phage, including λ and φ13 which only integrate at specific sites.
Transposable *P. aeruginosa* phage are commonplace, and include D3112 (Wang et al., 2004)
B3 (Braid et al., 2004) and LES φ4 (Winstanley et al., 2009). D3112 has been shown to
cause mutations in PAO1 through insertional inactivation (Rehmat & Shapiro, 1983).
However, the true extent of the impact of phage-mediated gene disruption on bacterial evolution remains poorly understood.

**Transduction:** Horizontal transfer of genetic material between bacterial genomes by a bacteriophage can occur by two different mechanisms. Both virulent and temperate phage types are capable of generalised transduction, which occurs during the lytic cycle of infection. Prior to cell lysis, phage heads are packaged with newly replicated phage genomes, but bacterial DNA can be mistakenly incorporated in place of the phage nucleic acid. Upon infection of another cell, the DNA is released into the cell cytoplasm and can potentially recombine with the host chromosome. 90% of temperate phage of the *S. Typhimurium* complex have been shown to perform generalised transduction in host bacterial populations (Ebel-Tsipis *et al.*, 1972, Schicklmaier & Schmieger, 1995). Generalised transduction of AMR genes has been observed during induction of a multi-drug resistant strain of *S. Typhimurium* using the veterinary antibiotic, carbadox (Bearson *et al.*, 2014). The recently characterised *P. aeruginosa* phage φPA3, originally isolated from sewage, is capable of infecting clinical CF isolates. It has been shown to transduce mutations in quorum sensing genes (*las* and *rhl*) in cultures of the lab strain PAO1 (Monson *et al.*, 2011).

Specialised transduction is mediated only by temperate phage, and occurs during imprecise excision of prophage from the bacterial genome, taking with it adjacent bacterial gene(s), which are transferred to another bacterial host upon lysogenic infection. Specialised transducing λ phage have been shown to transduce several important genes (Kirschbaum & Konrad, 1973, Jaskunas *et al.*, 1975, McEntee & Epstein, 1977, Hansen & von Meyenburg, 1979). Other examples of specialised transduction have been identified in *S. Typhimurium* (Chan *et al.*, 1972), *Bacillus subtilis* (Zahler *et al.*, 1977) and *P. aeruginosa* (Cavenagh & Miller, 1986).
Anchors for Chromosomal Rearrangements: Prophage can act as anchor points for chromosomal inversions and other major genomic rearrangements. Sequencing of a pathogenic *S. pyogenes* isolate identified two major chromosomal inversions, one of which was caused by homologous recombination between two related prophages, and the other was suggested to occur after a phage integration event which caused an “unbalancing” of the genome (Nakagawa *et al.*, 2003). There is evidence of a prophage-mediated chromosomal inversion in *E. faecium*, but despite the notion that major chromosomal rearrangements would have a negative impact on fitness, no such effect was detected (Lam *et al.*, 2012).

Polylysogeny

Polylysogeny, the carriage of multiple prophages, is a common feature of bacterial pathogens. The genomes of a wide range of *C. difficile* strains are highly plastic; carrying multiple prophages (Hargreaves *et al.*, 2015). Similarly, 18 co-existing prophages and 6 prophage-like elements have been identified in the chromosome of *E. coli* O157:H7 strain RIMD0509952 (Hayashi *et al.*, 2001). STEC are known to harbour several stx-encoding phage in the same chromosome, some of which exist in multiple copies, going against the classic lambdoid mechanisms of phage immunity. In this way, the expression of phage-encoded genes can be enhanced. For example multiple isogenic infections of *E. coli* by stx- phages have been shown to have a cumulative effect on the expression of Shiga toxin (Fogg *et al.*, 2012). There are several reports of polylysogenic *E. faecalis* that have been isolated from clinical samples. Strain V583 harbours seven different prophage-like elements, six of which constitute fully active, inducible, prophages that encode clear virulence traits and interact with each-other (Matos *et al.*, 2013). Similarly, the infection dynamics of multiple active LES prophages of *P. aeruginosa* have been described (Table 2). As with other
polylysogenic systems, the LES prophage sequences are mosaic in nature; LESφ3 is largely a hybrid of LESφ2 and LESφ5 (Figure 2). Of five active prophages, three exhibit productive infection of other *P. aeruginosa* strains. There is an interesting relationship between these prophages as LESφ2 confers immunity to infection by LESφ3 and LESφ4, which do not prevent infection by LESφ2. The LES prophages are also inducible with fluoroquinolone antibiotics and exhibit a hierarchical nature, with LESφ2 density being consistently higher than the other LES phage *in vivo* and *in vitro* (James *et al.*, 2012).

Experimental evolution experiments have begun to explore the cost/benefits of polylysogeny and the interactions between co-habiting prophages. Carriage of two LES prophages has been shown to confer a competitive advantage over single lysogens during mixed infection in wax moth larvae (Burns *et al.*, 2015). Within host competition of 11 different *E. coli* prophages has also suggested a hierarchical relationship during stressful conditions. In these experiments, double lysogens were exposed to the potent inducing agent, mitomycin C. In most cases, the prophage with the fastest response to induce the lytic cycle showed a competitive advantage (Refardt, 2011). These studies suggest that interactions between prophages and diversity in phage immunity mechanisms can also alter the course of bacterial adaptation.

**CRISPR Immunity to temperate phage**

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are widespread in bacterial genomes and act as an active defence mechanism to protect against bacteriophage infection (Barrangou *et al.*, 2007). This mechanism of protection against virulent phage has been well documented. However, the relationship between CRISPR and temperate phage is less clear. Several reports suggest that CRISPR systems are negatively correlated with
lysogeny and there is evidence that *E. coli* CRISPRs prevent both lysogenic infection and
induction of prophages (Fogg *et al.*, 2010). Others have demonstrated an interaction between
CRISPR and the prophage DMS3. The presence of both together has been shown to inhibit
biofilm formation and swarming in *P. aeruginosa* (Zegans *et al.*, 2009). CRISPR spacers
with 100% identity to temperate phage sequences are widespread amongst clinical isolates of
*P. aeruginosa*, including the LES (Cady *et al.*, 2011). The overall effects of CRISPR
evolution, in response to temperate bacteriophages, on bacterial adaptation require further
exploration.

**Outlook**

The contribution of prophage to the success of their bacterial hosts during infection
has been under studied, especially in the case of prophage that do not contribute a clear
phenotype such as toxin production. A wealth of readily available whole-genome sequence
data has now enabled the identification of previously un-discovered prophages and cryptic
prophage elements, revealing their abundance in an array of different environments.

However, biological understanding of the roles of the many “unknown” proteins harboured
by the prophages remains some way behind the generation of these sequence data. In addition
to this, there is a lack of functional studies into the mechanistic contributions of these phage
to the host. Since temperate phage can switch between lysis and lysogeny, they are
particularly important in the evolutionary dynamics of bacterial populations, leading to a
complex interplay between symbiotic and competitive relationships of multiple interacting
phage and their hosts. The additional influence of lysis-inducing antibiotic treatments can
potentially change the trajectory of bacterial adaptation in the host environment. An
understanding of these infection dynamics in vivo is needed to develop novel strategies for managing chronic bacterial infection.

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by fluoroquinolones in Streptococcus pneumoniae: implications for emergence of resistance

efficiently package various bacterial genes and mobile genetic elements including SCCmec


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**Figure 1. The temperate phage lifecycle.**

A: Lysogeny occurs when the phage DNA integrates into the bacterial genome. Here it is described as a prophage. Prophages replicate along with the bacterial cell. Cell stress such as DNA damage can result in the prophage entering the lytic cycle leading to phage replication and release following bacterial cell lysis. B: Scanning electron microscope image of an *E. coli* cell under-going lysis triggered by the stx-phage Φ24B (*James C. E.* un-published).
Table 1: Prophage associated-genes involved in bacterial virulence.

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<tr>
<td>C. diptheriae</td>
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<td>Diptheria toxin (tox) Cytotoxin</td>
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<td>E. coli</td>
<td>Stx</td>
<td>Shiga toxin (stx1, stx2), cytotoxins stk - Affects signal transduction</td>
<td>(Wagner et al. 2001) (Plunkett et al. 1999) (Lavigne and Blanc-Potard 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TTSS Effectors cif, espI/nleA, espI, espK, espEU/tccP, nleI</td>
<td></td>
</tr>
<tr>
<td>λ</td>
<td></td>
<td>lom - binding to epithelial cells</td>
<td>(Vica Pacheco et al. 1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bor - Outer membrane protein that aids bacterial immune evasion.</td>
<td>(Barondess and Beckwith 1995)</td>
</tr>
<tr>
<td>CP-933C</td>
<td></td>
<td>Cryptic phage regulates TTSS</td>
<td>(Flockhart et al. 2012)</td>
</tr>
<tr>
<td>S. enterica</td>
<td>φSopE</td>
<td>TTSS effector (sopE) promotes invasion of epithelial cells.</td>
<td>(Mirold et al. 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gipA, gogB - survival and growth in Peyer’s patches.</td>
<td>(Stanley et al. 2000)</td>
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<tr>
<td>Gifsy-1</td>
<td></td>
<td>sodC1, SseI - survival in macrophages</td>
<td>(Figueroa-Bossi et al. 2001)</td>
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<tr>
<td>Gifsy-2</td>
<td></td>
<td>sspHI - TTSS effector</td>
<td>(Ehrbar and Hardt 2005)</td>
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<td>P. aeruginosa</td>
<td>D3</td>
<td>Altered outer membrane properties reduces phagocytosis</td>
<td>(Holloway and Cooper 1962)</td>
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<tr>
<td>S. mitis</td>
<td>SM1</td>
<td>pblA and pblB - Platelet binding</td>
<td>(Bensing et al. 2001)</td>
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<td>C. jejuni</td>
<td>CJIE1</td>
<td>Increased adherence and invasion</td>
<td>(Clark et al. 2012)</td>
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<tr>
<td>V. cholerae</td>
<td>CTX</td>
<td>ctx - Cytotoxin</td>
<td>(Faruque et al. 1998)</td>
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<tr>
<td>LES prophage</td>
<td>Characteristics</td>
<td>N° of genes</td>
<td>Related phages in reference strain PAO1</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------</td>
<td>-------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>φ1</td>
<td>Defective prophage, predicted to encode pyocin R2</td>
<td>19</td>
<td>Defective prophage gene cluster encoding pyocin R2</td>
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<tr>
<td>φ2</td>
<td>Active inducible prophage, encodes integrase for site-specific integration</td>
<td>44</td>
<td>None</td>
</tr>
<tr>
<td>φ3</td>
<td>Active inducible prophage, encodes integrase for site-specific integration</td>
<td>53</td>
<td>None</td>
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<tr>
<td>φ4</td>
<td>Active inducible prophage, encodes transposase. Capable of random integration.</td>
<td>48</td>
<td>None</td>
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<tr>
<td>φ5</td>
<td>Active inducible prophage, encodes integrase</td>
<td>65</td>
<td>None</td>
</tr>
<tr>
<td>φ6</td>
<td>Active inducible prophage, encodes integrase</td>
<td>12</td>
<td>Pf4 filamentous phage implicated in biofilm dispersal</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of LES prophage
**Figure 2.** Mosaicism of LES prophages.

Circos map (Krzywinski et al. 2009) depicting an alignment of five prophage sequences from the Liverpool Epidemic Strain of *Pseudomonas aeruginosa* (EMBL accession number FM209186) using the Artemis Comparison Tool (Carver et al. 2005). Each coloured segment of the circumference represents a LES prophage genome. Ribbons that link prophage regions show regions of sequence homology.
The Role of Temperate Bacteriophages in Bacterial Infection

Temperate bacterial viruses can lyse bacterial cells (shown) or incorporate into their genomes, often providing key adaptations that are important for infection.