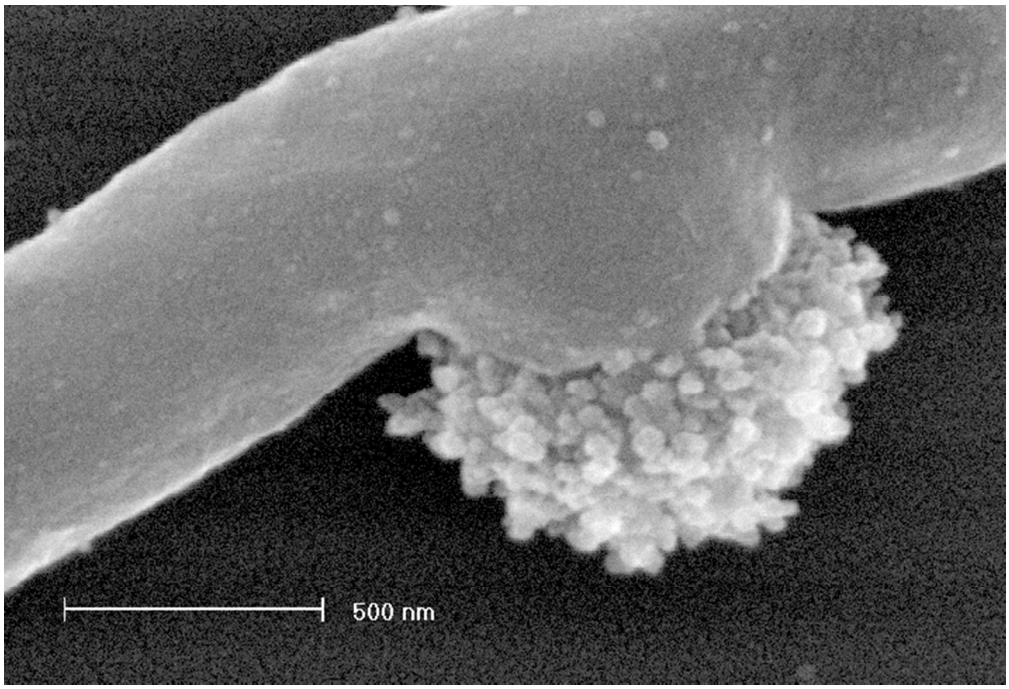
OXFORD UNIVERSITY PRESS | FEMS Microbiology Letters

http://mc.manuscriptcentral.com/fems

The Role of Temperate Bacteriophages in Bacterial Infection

Journal:	*FEMS Microbiology Letters*
Manuscript ID	FEMSLE-15-11-0981.R1
Manuscript Type:	Minireview
Date Submitted by the Author:	n/a
Complete List of Authors:	Davies, Emily; University of Liverpool, Institute of Infection and Global Health Winstanley, Craig; University of Liverpool, Institute of Infection and Global Health Fothergill, Joanne; University of Liverpool, Institute of Infection and Global Health James, Chloe; University of Salford, School of Environment and Life Sciences
Keywords:	Temperate Bacteriophage, Bacterial Pathogens, Infection, Adaptation
All articles in FEMS Microbiology Letters are published under one of eight subject sections. Please select the most appropriate subject category for your submission from the drop down list:	Pathogens & Pathogenicity (Editor: Arnoud van Vliet)



623x472mm (29 x 26 DPI)

The Role of Temperate Bacteriophages in Bacterial Infection

Emily V. Davies[1], Craig Winstanley[1], Joanne L. Fothergill[1*] and Chloe E. James[2*]

[1] Institute of Infection and Global Health, University of Liverpool, UK

[2] School of Environment and Life Sciences, University of Salford, UK

*These authors contributed equally to this manuscript.

Corresponding Author: Chloe E. James

Address: School of Environment and Life Sciences, G28 Peel Building, University of Salford, Manchester M5 4WT

Email: c.james@salford.ac.uk

Tel: 0161 295 2171

Key words: Temperate Bacteriophage; Bacterial Pathogens; Infection; Adaptation

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 *cI*, 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 in to 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

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 diptheriae* 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 stx_2 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 (Quirós *et al.*, 2014), and the lungs of individuals with CF (Fancello *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 $bla_{\text{CMY-2}}$ 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-β-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 *in vivo* following antibiotic treatment of mice. Interestingly, they detected enrichment of disparate mechanisms to resist

both the administered drug and un-related 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 x 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 gastro-intestinal 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 *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 supress 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.

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.

Funding

This work was supported by the Wellcome Trust (CEJ, CW) [grant number 089215/Z/09/Z] and an Early Career Fellowship from the Leverhulme Trust (JLF).

References

Aanaes K, Rickelt LF, Johansen HK, *et al.* (2011) Decreased mucosal oxygen tension in the maxillary sinuses in patients with cystic fibrosis. *J. Cystic Fibrosis* **10**: 114-20.

Barondess JJ & Beckwith J (1995) *bor* gene of phage lambda, involved in serum resistance, encodes a widely conserved outer membrane lipoprotein. *J. Bacteriology* **177**: 1247-53.

Barrangou R, Fremaux C, Deveau H, *et al.* (2007) CRISPR provides acquired resistance against viruses in prokaryotes. *Science* **315**: 1709-12.

Bearson BL, Allen HK, Brunelle BW, *et al.* (2014) The agricultural antibiotic carbadox induces phage-mediated gene transfer in *Salmonella*. *Frontiers in Microbiol.* **5**.

Bensing BA, Siboo IR & Sullam PM (2001) Proteins PblA and PblB of *Streptococcus mitis,* which promote binding to human platelets, are encoded within a lysogenic bacteriophage. *Infect. Immun.* **69**: 6186-92.

Bobay LM, Touchon M & Rocha EP (2014) Pervasive domestication of defective prophages by bacteria. *PROC. NATL. ACAD. SCI. U.S.A.* **111**: 12127-32.

Bondy-Denomy J & Davidson AR (2014) When a virus is not a parasite: the beneficial effects of prophages on bacterial fitness. *J. Microbiol.* **52**: 235-42.

Braid MD, Silhavy JL, Kitts CL, Cano RJ & Howe MM (2004) Complete genomic sequence of bacteriophage B3, a Mu-like phage of *Pseudomonas aeruginosa. J. Bacteriol* **186**: 6560-74.

Breitbart M, Hewson I, Felts B, *et al.* (2003) Metagenomic analyses of an uncultured viral community from human feces. *J. Bacteriol.* **185**: 6220-3.

Bukhari A & Zipser D (1972) Random insertion of Mu-1 DNA within a single gene. *Nature* **236**: 240-3.

Burns N, James CE & Harrison E (2015) Polylysogeny magnifies competitiveness of a bacterial *pathogen in vivo*. *Evolutionary Applications* **8**: 346-51.

Busby B, Kristensen DM & Koonin EV (2013) Contribution of phage-derived genomic islands to the virulence of facultative bacterial pathogens. *Environ. Microbiol.* **15**: 307-12.

Cabral DA, Loh BA & Speert DP (1987) Mucoid *Pseudomonas aeruginosa* resists nonopsonic phagocytosis by human neutrophils and macrophages. *Pediatric Res.* **22**: 429-31.

Cady KC, White AS, Hammond JH, *et al.* (2011) Prevalence, conservation and functional analysis of *Yersinia* and *Escherichia* CRISPR regions in clinical *Pseudomonas aeruginosa* isolates. *Microbiol.* **157**: 430-7.

Carrolo M, Frias MJ, Pinto FR, *et al.* (2010) Prophage spontaneous activation promotes DNA release enhancing biofilm formation in *Streptococcus pneumoniae*. *PloSONE* **5**: e15678.

Carver TJ, Rutherford KM, Berriman M, *et al.* (2005) ACT: the Artemis Comparison Tool. *Bioinformatics* **21**: 3422-3.

Casas V & Maloy S (2011) Role of bacteriophage-encoded exotoxins in the evolution of bacterial pathogens. *Future Microbiol.* **6**: 1461-73.

Cavenagh MM & Miller RV (1986) Specialized transduction of *Pseudomonas aeruginosa* PAO by bacteriophage D3. *J. Bacteriol.* **165**: 448-52.

Chan RK, Botstein D, Watanabe T & Ogata Y (1972) Specialized transduction of tetracycline resistance by phage P22 in *Salmonella typhimurium*: II. Properties of a high-frequency-transducing lysate. *Virology* **50**: 883-98.

Cirz RT, O'Neill BM, Hammond JA, *et al.* (2006) Defining the *Pseudomonas aeruginosa* SOS response and its role in the global response to the antibiotic ciprofloxacin. *J. Bacteriol.* **188**: 7101-10.

Clark CG, Grant CC, Pollari F, *et al.* (2012) Effects of the *Campylobacter jejuni* CJIE1 prophage homologs on adherence and invasion in culture, patient symptoms, and source of infection. *BMC microbiology* **12**: 269.

Coleman D, Knights J, Russell R, *et al.* (1991) Insertional inactivation of the *Staphylococcus aureus* β-toxin by bacteriophage φ13 occurs by site-and orientation-specific integration of the φ 13 genome. *Mol. Microbiol.* **5**: 933-39.

Colomer-Lluch M, Jofre J & Muniesa M (2011) Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. *PLoSONE* **6**.

Dhillon TS, Dhillon EK, Chau HC, *et al.* (1976) Studies on bacteriophage distribution: virulent and temperate bacteriophage content of mammalian feces. *Appl. Environ. Microbiol* **32**: 68-74.

Ebel-Tsipis J, Botstein D & Fox MS (1972) Generalized transduction by phage P22 in *Salmonella typhimurium*: I. Molecular origin of transducing DNA. *J. Mol. Biol.* **71**: 433-48.

Ehrbar K & Hardt WD (2005) Bacteriophage-encoded type III effectors in *Salmonella enterica* subspecies 1 serovar Typhimurium. *Infection, genetics and evolution : J. Mol. Epidemiol. and Evol. Gen. Infect. Dis.* **5**: 1-9.

Fancello L, Desnues C, Raoult D & Rolain JM (2011) Bacteriophages and diffusion of genes encoding antimicrobial resistance in cystic fibrosis sputum microbiota. *J. Antimicrob. Chemother.* **66**: 2448-54.

Faruque SM, Asadulghani, Alim AR, *et al.* (1998) Induction of the lysogenic phage encoding cholera toxin in naturally occurring strains of toxigenic *Vibrio cholerae* O1 and O139. *Infect. Immun.* **66**: 3752-7.

Fischer-Fantuzzi, L., and Calef, E. (1964) A type of A prophage unable to confer immunity. *Virology* **23**: 209-216.

Figueroa-Bossi N, Uzzau S, Maloriol D & Bossi L (2001) Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in *Salmonella*. *Mol. Microbiol.* **39**: 260-71.

Flockhart AF, Tree JJ, Xu X*, et al.* (2012) Identification of a novel prophage regulator in *Escherichia coli* controlling the expression of type III secretion. *Mol. Microbiol.* **83**: 208-23.

Fogg PC, Allison HE, Saunders JR & McCarthy AJ (2010) Bacteriophage lambda: a paradigm revisited. *J. Virol.* **84**: 6876-9.

Fogg PC, Saunders JR, McCarthy AJ & Allison HE (2012) Cumulative effect of prophage burden on Shiga toxin production in *Escherichia coli*. *Microbiol.* **158**: 488-97.

Fortier LC & Sekulovic O (2013) Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence* **4**: 354-65.

Fothergill JL, Mowat E, Walshaw MJ, *et al.* (2011) Effect of antibiotic treatment on bacteriophage production by a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa*. *Antimicrob. Gen. Chemother.* **55**: 426-8.

Frobisher M & Brown JH (1927) Transmissible toxigenicity of streptococci. *Bull Johns Hopkins Hosp* **41**: 167-73.

Furuse K, Osawa S, Kawashiro J, *et al.* (1983) Bacteriophage distribution in human faeces: continuous survey of healthy subjects and patients with internal and leukaemic diseases. *J. Gen. Virol.* **64**: 2039-43.

Gobert AP, Vareille M, Glasser A-L, *et al.* (2007) Shiga Toxin Produced by Enterohemorrhagic *Escherichia coli* Inhibits PI3K/NF-κB Signaling Pathway in Globotriaosylceramide-3-Negative Human Intestinal Epithelial Cells. *J. Immunol.* **178**: 8168-74.

Groman NB (1953) Evidence for the induced nature of the change from nontoxigenicity to toxigenicity in *Corynebacterium diphtheriae* as a result of exposure to specific bacteriophage. *J. Bacteriol.* **66**: 184-91.

Groman NB (1955) Evidence for the active role of bacteriophage in the conversion of nontoxigenic *Corynebacterium diphtheriae* to toxin production. *J. Bacteriol.* **69**: 9.

Hacker J & Carniel E (2001) Ecological fitness, genomic islands and bacterial pathogenicity. *EMBO Reports* **2**: 376-81.

Hansen FG & von Meyenburg K (1979) Characterization of the *dnaA, gyrB* and other genes in the *dnaA* region of the *Escherichia coli* chromosome on specialized transducing phages λtna. *Mol. Gen. Genetics* **175**: 135-44.

Hargreaves, K. R., Otieno, J. R., Thanki, A., *et al.,* (2015). As Clear as Mud? Determining the Diversity and Prevalence of Prophages in the Draft Genomes of Estuarine Isolates of *Clostridium difficile*. *Genome Biol. and Evol.* **7:** 1842–55.

Hayashi T, Makino K, Ohnishi M*, et al.* (2001) Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res.* **8**: 11-22.

Hentzer M, Teitzel GM, Balzer GJ, *et al.* (2001) Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. *J. Bacteriol.* **183**: 5395-5401.

Holloway BW & Cooper GN (1962) Lysogenic conversion in *Pseudomonas aeruginosa. J. Bacteriol.* **84**: 1321-4.

Holmes RK & Barksdale L (1969) Genetic analysis of tox+ and tox- bacteriophages of Corynebacterium diphtheriae. *J. Virol.* **3**: 586-98.

Hraiech S, Bregeon F & Rolain JM (2015) Bacteriophage-based therapy in cystic fibrosis-associated *Pseudomonas aeruginosa* infections: rationale and current status. *Drug Design, Development and Therapy* **9**: 3653-63.

James CE, Stanley KN, Allison HE, Flint HJ, Stewart CS, Sharp RJ, Saunders JR and McCarthy AJ (2001) Lytic and lysogenic infection of diverse *Escherichia coli* and *Shigella* strains with a verocytotoxigenic bacteriophage. *Appl. Environ. Microbiol.* **67:** 4335-7

James CE, Fothergill JL, Kalwij H, *et al.* (2012) Differential infection properties of three inducible prophages from an epidemic strain of *Pseudomonas aeruginosa*. *BMC Microbiol.* **12**: 216.

James CE, Davies EV, Fothergill JL, *et al.* (2015) Lytic activity by temperate phages of *Pseudomonas aeruginosa* in long-term cystic fibrosis chronic lung infections. *ISME J.* **9**: 1391-8.

Jaskunas SR, Lindahl L & Nomura M (1975) Specialized transducing phages for ribosomal protein genes of *Escherichia coli. PROC. NATL. ACAD. SCI. U.S.A.* **72**: 6-10.

Juhala RJ, Ford ME, Duda RL, *et al.* (2000) Genomic sequences of bacteriophages HK97 and HK022: pervasive genetic mosaicism in the lambdoid bacteriophages. *J. Mol. Biol* **299**: 27-51.

Kim MS, Park EJ, Roh SW & Bae JW (2011) Diversity and abundance of single-stranded DNA viruses in human feces. *Appl Environ Microbiol* **77**: 8062-8070.

Kirschbaum JB & Konrad EB (1973) Isolation of a specialized lambda transducing bacteriophage carrying the beta subunit gene for *Escherichia coli* ribonucleic acid polymerase. *J. Bacteriol.* **116**: 517-26.

Krzywinski M, Schein J, Birol I, *et al.* (2009) Circos: an information aesthetic for comparative genomics. *Genome Res.* **19**: 1639-45.

Labrie SJ, Samson JE & Moineau S (2010) Bacteriophage resistance mechanisms. *Nat. Rev. Microbiol.* **8**: 317-27.

Lam MM, Seemann T, Bulach DM, *et al.* (2012) Comparative analysis of the first complete *Enterococcus faecium* genome. *J. Bacteriol.* **194**: 2334-41.

Lavigne JP & Blanc-Potard AB (2008) Molecular evolution of *Salmonella enterica* serovar Typhimurium and pathogenic *Escherichia coli*: from pathogenesis to therapeutics. *Infection, genetics and evolution: J. Mol. Epidemiol. Evol. Gen. Infect. Dis.* **8**: 217-26.

Lee C & Iandolo J (1986) Lysogenic conversion of staphylococcal lipase is caused by insertion of the bacteriophage L54a genome into the lipase structural gene. *J. Bacteriol.* **166**: 385-91.

Lee, Y-D and Park, J-H (2015) Phage Conversion for β-lactam Antibiotics Resistance of *Staphylococcus aureus* from Foods. *J. Microbiol and Biotech.* **25:** (9)

Lemieux AA, Jeukens J, Kukavica-Ibrulj I, *et al.* (2015) Genes Required for Free Phage Production are Essential for *Pseudomonas aeruginosa* Chronic Lung Infections. *J. Infect. Dis*.

Levin BR & Svanborg Eden C (1990) Selection and evolution of virulence in bacteria: An ecumenical excursion and modest suggestion. *Parasitol.* **100**: S103-S115.

Lieb M (1953) The establishment of lysogenicity in *Escherichia coli*. *J. Bacteriol.* **65**: 642-51.

López E, Domenech A, Ferrándiz MJ, *et al*... de la Campa AG **(2014)** Induction of prophages by fluoroquinolones in *Streptococcus pneumoniae*: implications for emergence of resistance in genetically-related clones. PLoSONE. **9:** e94358

Maslanova I, Doskar J, Varga M, *et al.* (2013) Bacteriophages of *Staphylococcus aureus* efficiently package various bacterial genes and mobile genetic elements including SCCmec with different frequencies. *Environ. Microbiol. Rep.* **5**: 66-73.

Matos RC, Lapaque N, Rigottier-Gois L, *et al.* (2013) *Enterococcus faecalis* Prophage Dynamics and Contributions to Pathogenic Traits. *PLoS Genetics* **9**: e1003539.

Matsushiro A, Sato K, Miyamoto H, *et al.* (1999) Induction of prophages of enterohemorrhagic *Escherichia coli* O157:H7 with norfloxacin. *J. Bacteriol.* **181**: 2257-60.

Mazaheri Nezhad Fard R, Barton MD & Heuzenroeder MW (2011) Bacteriophage-mediated transduction of antibiotic resistance in enterococci. *Lett. Appl. Microbiol.* **52**: 559-64.

McEntee K & Epstein W (1977) Isolation and characterization of specialized transducing bacteriophages for the recA gene of *Escherichia coli*. *Virology* **77**: 306-18.

Meessen-Pinard M, Sekulovic O & Fortier LC (2012) Evidence of *in vivo* prophage induction during *Clostridium difficile* infection. *Appl. Environ. Microbiol.* **78**: 7662-70.

Miller RV & Renta Rubero VJ (1984) Mucoid conversion by phages of *Pseudomonas aeruginosa* strains from patients with cystic fibrosis. *J. Clin. Microbiol* **19**: 717-719.

Mirold S, Rabsch W, Rohde M, *et al.* (1999) Isolation of a temperate bacteriophage encoding the type III effector protein SopE from an epidemic *Salmonella typhimurium* strain. *PROC. NATL. ACAD. SCI. U.S.A.* **96**: 9845-9850.

Mizutani S, Nakazono N & Sugino Y (1999) The so-called chromosomal verotoxin genes are actually carried by defective prophages. *DNA Res.* **6**: 141-3.

Modi, S. R., Lee, H. H., Spina, C. S., & Collins, J. J. (2013). Antibiotic Treatment Expands the Resistance Reservoir and Ecological Network of the Phage Metagenome. *Nature* **499:** 219–222.

Monson R, Foulds I, Foweraker J, *et al.* (2011) The *Pseudomonas aeruginosa* generalized transducing phage φPA3 is a new member of the φKZ-like group of 'jumbo'phages, and infects model laboratory strains and clinical isolates from cystic fibrosis patients. *Microbiol.* **157**: 859-867.

Nakagawa I, Kurokawa K, Yamashita A, *et al.* (2003) Genome sequence of an M3 strain of *Streptococcus pyogenes* reveals a large-scale genomic rearrangement in invasive strains and new insights into phage evolution. *Genome Res* **13**: 1042-55.

Nassar FJ, Rahal EA, Sabra A & Matar GM (2013) Effects of subinhibitory concentrations of antimicrobial agents on *Escherichia coli* O157:H7 Shiga toxin release and role of the SOS response. *Foodborne Pathogens and Disease* **10**: 805-12.

Ohnishi M, Terajima J, Kurokawa K, *et al.* (2002) Genomic diversity of enterohemorrhagic *Escherichia coli* O157 revealed by whole genome PCR scanning. *PROC. NATL. ACAD. SCI. U.S.A.* **99**: 17043-8.

Ojeniyi B, Birch-Andersen A, Mansa B, *et al.* (1991) Morphology of *Pseudomonas aeruginosa* phages from the sputum of cystic fibrosis patients and from the phage typing set. An electron microscopy study. *APMIS* **99**: 925-30.

Parsley, L. C., Consuegra, E. J., Kakirde, K. S., *et al.* (2010) Identification of Diverse Antimicrobial Resistance Determinants Carried on Bacterial, Plasmid, or Viral Metagenomes from an Activated Sludge Microbial Assemblage. *Appl. Environ. Microbiol.* **7:** 3753–7

Plunkett G, 3rd, Rose DJ, Durfee TJ & Blattner FR (1999) Sequence of Shiga toxin 2 phage 933W from *Escherichia coli* O157:H7: Shiga toxin as a phage late-gene product. *J. Bacteriol.* **181**: 1767-78.

Pride DT, Salzman J, Haynes M, *et al.* (2012) Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome. *ISME J* **6**: 915-26.

Ptashne M (2004) Two "what if" experiments. *Cell* **116**: S71-72

Quirós P, Colomer-Lluch M, Martínez-Castillo A, *et al.* (2014) Antibiotic Resistance Genes in the Bacteriophage DNA Fraction of Human Fecal Samples. *Antimicrob. Agents Chemother.* **58**: 606-9.

Refardt D (2011) Within-host competition determines reproductive success of temperate bacteriophages. *ISME J* **5**: 1451-60.

Refardt D & Rainey PB (2010) Tuning a genetic switch: experimental evolution and natural variation of prophage induction. *Evolution* **64**: 1086-97.

Rehmat S & Shapiro JA (1983) Insertion and replication of the *Pseudomonas aeruginosa* mutator phage D3112. *Mol. Gen. Genetics* **192**: 416-23.

Reyes A, Semenkovich NP, Whiteson K, *et al.* (2012) Going viral: next-generation sequencing applied to phage populations in the human gut. *Nat. Rev. Microbiol.* **10**: 607-17.

Rice SA, Tan CH, Mikkelsen PJ, *et al.* (2009) The biofilm life cycle and virulence of *Pseudomonas aeruginosa* are dependent on a filamentous prophage. *ISME J.* **3**: 271-82.

Robinson CM, Sinclair JF, Smith MJ & O'Brien AD (2006) Shiga toxin of enterohemorrhagic *Escherichia coli* type O157:H7 promotes intestinal colonization. *PROC. NATL. ACAD. SCI. U.S.A.* **103**: 9667-72.

Rohwer F & Thurber RV (2009) Viruses manipulate the marine environment. *Nature* **459**: 207-12.

Rolain JM, Fancello L, Desnues C & Raoult D (2011) Bacteriophages as vehicles of the resistome in cystic fibrosis. *J. Antimicrob. Chemother.* **66**: 2444-7.

Roux, S., Enault, F., Hurwitz, B. L., & Sullivan, M. B. (2015). VirSorter: mining viral signal from microbial genomic data. *PeerJ*, *3*, e985

Schicklmaier P & Schmieger H (1995) Frequency of generalized transducing phages in natural isolates of the *Salmonella typhimurium* complex. *Appl. Environ. Microbiol.* **61**: 1637-40.

Schuch R & Fischetti VA (2009) The secret life of the anthrax agent *Bacillus anthracis*: bacteriophage-mediated ecological adaptations. *PloS ONE* **4**: e6532.

Stanley TL, Ellermeier CD & Slauch JM (2000) Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar typhimurium survival in Peyer's patches. *J. Bacteriol.* **182**: 4406-13.

Stewart FM & Levin BR (1984) The population biology of bacterial viruses: why be temperate. *Theoretical Population Biology* **26**: 93-117.

Tang F, Zhang W & Lu C (2013) Lysogenic *Streptococcus suis* Isolate SS2-4 Containing Prophage SMP Showed Increased Mortality in Zebra Fish Compared to the Wild-Type Isolate. *PLoS ONE* **8**.

Taylor AL (1963) Bacteriophage-induced mutation in *Escherichia coli*. *PROC. NATL. ACAD. SCI. U.S.A.* **50**: 1043.

Vica Pacheco S, Garcia Gonzalez O & Paniagua Contreras GL (1997) The *lom* gene of bacteriophage lambda is involved in *Escherichia coli* K12 adhesion to human buccal epithelial cells. *FEMS Microbiol. Lett.* **156**: 129-32.

Wagner PL, Neely MN, Zhang X, *et al.* (2001) Role for a phage promoter in Shiga toxin 2 expression from a pathogenic *Escherichia coli* strain. *J. Bacteriol.* **183**: 2081-5.

Wagner PL, Livny J, Neely MN, (2002) Bacteriophage control of Shiga toxin 1 production and release by *Escherichia coli*. *Mol. Microbiol* **44**: 957-70.

Wang PW, Chu L & Guttman DS (2004) Complete sequence and evolutionary genomic analysis of the *Pseudomonas aeruginosa* transposable bacteriophage D3112. *J. Bacteriol.* **186**: 400-10.

Wang X, Kim Y, Ma Q, *et al.* (2010) Cryptic prophages help bacteria cope with adverse environments. *Nature Communications* **1**: 147.

Willner D, Furlan M, Haynes M, *et al.* (2009) Metagenomic analysis of respiratory tract DNA viral communities in cystic fibrosis and non-cystic fibrosis individuals. *PLoS ONE* **4**: e7370.

Willner D, Furlan M, Schmieder R, *et al.* (2011) Metagenomic detection of phage-encoded platelet-binding factors in the human oral cavity. *Proc. Natl. Acad. Sci. U.S.A.* **108** S1: 4547-4553.

Winstanley C, Langille MG, Fothergill JL, *et al.* (2009) Newly introduced genomic prophage islands are critical determinants of in vivo competitiveness in the Liverpool Epidemic Strain of *Pseudomonas aeruginosa*. *Genome Res* **19**: 12-23.

Winter C, Bouvier T, Weinbauer MG & Thingstad TF (2010) Trade-offs between competition and defense specialists among unicellular planktonic organisms: the "killing the winner" hypothesis revisited. *Microbiol. and Mol. Biol. Rev.* **74**: 42-57.

Zahler S, Korman R, Rosenthal R & Hemphill H (1977) *Bacillus subtilis* bacteriophage SPbeta: localization of the prophage attachment site, and specialized transduction. *J. Bacteriol.* **129**: 556-8.

Zegans ME, Wagner JC, Cady KC, *et al.* (2009) Interaction between bacteriophage DMS3 and host CRISPR region inhibits group behaviors of *Pseudomonas aeruginosa*. *J. Bacteriol.* **191**: 210-19.

Zhang Y & LeJeune JT (2008) Transduction of bla(CMY-2), tet(A), and tet(B) from Salmonella enterica subspecies enterica serovar Heidelberg to *S. Typhimurium*. *Vet. Microbiol.* **129**: 418-25.

Figures and Tables

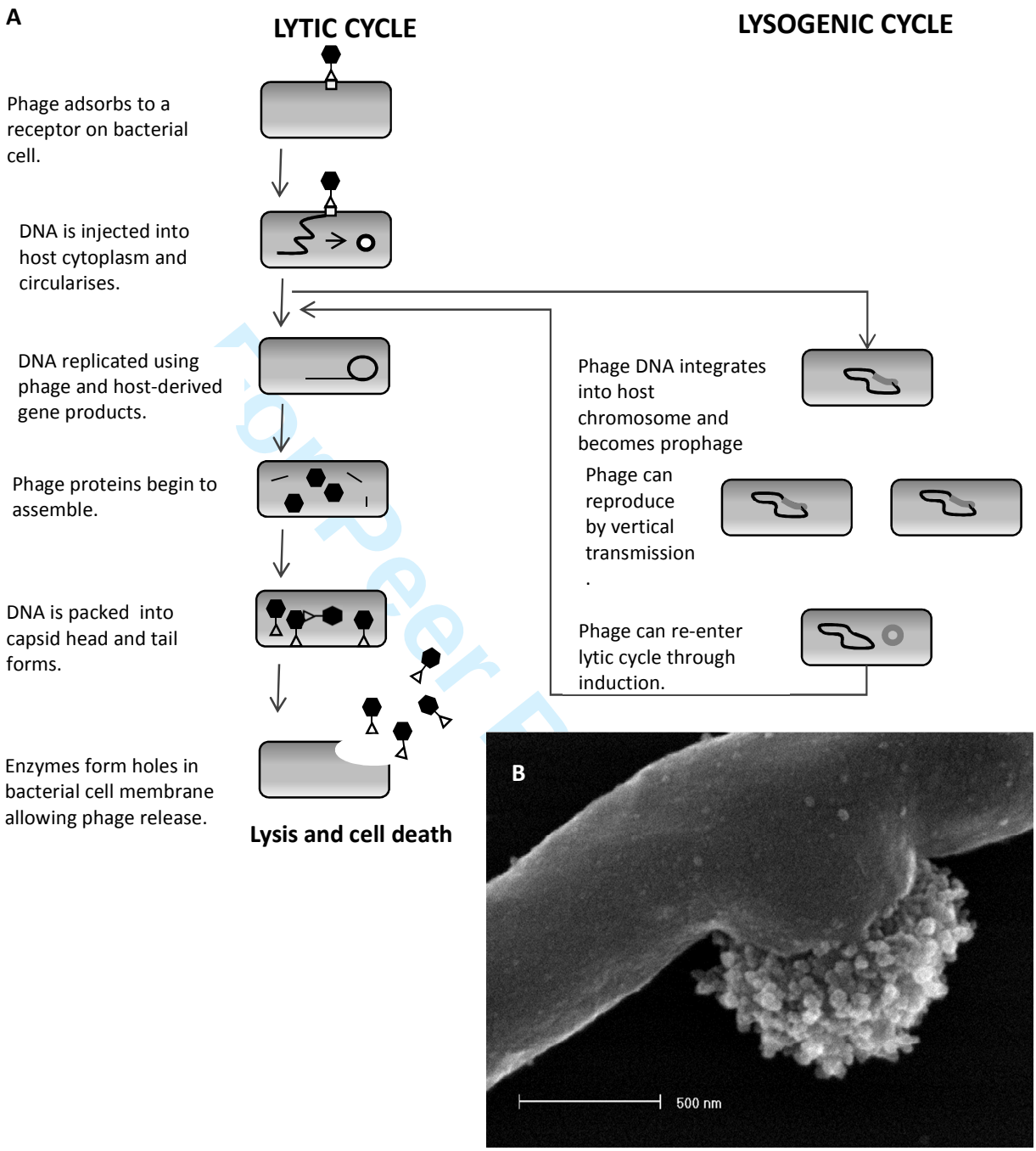


Figure 1. The temperate phage lifecycle.

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.

Bacteria	Phage	Phage-encoded virulence gene(s)	Reference
C. diptheriae	Beta	Diptheria toxin (*tox*) Cytotoxin	(Holmes and Barksdale 1969)
E. coli	Stx	Shiga toxin (stx_1, stx_2), cytotoxins *stk* - Affects signal transduction TTSS Effectors *cif, espI/nleA, espI, espK, espEU/tccP, nleI*	(Wagner et al. 2001) (Plunkett et al. 1999) (Lavigne and Blanc-Potard 2008)
	λ	*lom* - binding to epithelial cells *bor* - Outer membrane protein that aids bacterial immune evasion.	(Vica Pacheco et al. 1997) (Barondess and Beckwith 1995)
	CP-933C	Cryptic phage regulates TTSS	(Flockhart et al. 2012)
S. enterica	ϕSopE	TTSS effector (*sopE*) promotes invasion of epithelial cells.	(Mirold et al. 1999)
	Gifsy-1	*gipA, gogB* - survival and growth in Peyer's patches.	(Stanley et al. 2000)
	Gifsy-2	*sodC1, SseI* - survival in macrophages	(Figueroa-Bossi et al. 2001)
	Gifsy-3	*sspHI* - TTSS effector	(Ehrbar and Hardt 2005)
P. aeruginosa	D3	Altered outer membrane properties reduces phagocytosis	(Holloway and Cooper 1962)
S. mitis	SM1	*pblA* and *pblB* - Platelet binding	(Bensing et al. 2001)
C. jejuni	CJIE1	Increased adherence and invasion	(Clark et al. 2012)
V. cholerae	CTX	*ctx* - Cytotoxin	(Faruque et al. 1998)

Table 2. Characteristics of LES prophage

LES prophage	Characteristics	N° of genes	Related phages in reference strain PAO1	Known related phages	Interaction with other LES phages
ϕ1	Defective prophage, predicted to encode pyocin R2	19	Defective prophage gene cluster encoding pyocin R2	Pyocin gene clusters predicted to have evolved from phage tail genes	Unknown
ϕ2	Active inducible prophage, encodes integrase for site-specific integration	44	None	None	Confers resistance to infection by ϕ3 and ϕ4
ϕ3	Active inducible prophage, encodes integrase for site-specific integration	53	None	Homologous regions in LESϕ2 and LESϕ5	Shares same cI gene region as ϕ2
ϕ4	Active inducible prophage, encodes transposase. Capable of random integration.	48	None	D3112	Present in 100% LES isolates
ϕ5	Active inducible prophage, encodes integrase	65	None	D3	Present in only a small proportion of LES isolates
ϕ6	Active inducible prophage, encodes integrase	12	Pf4 filamentous phage implicated in biofilm dispersal	Filamentous phage Pf1 (Family *Inoviridae*)	Unknown

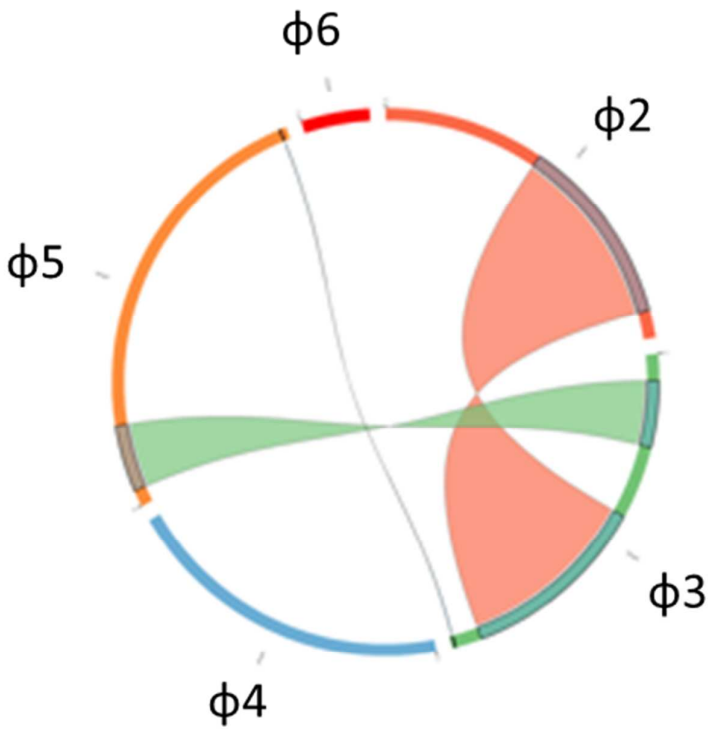
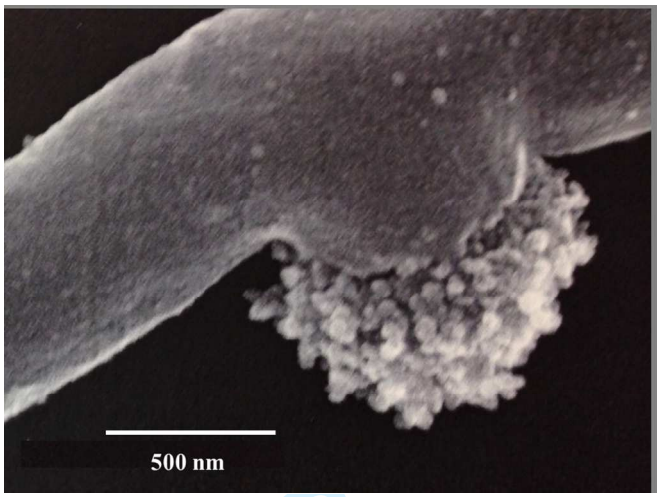


Figure 2. Mosaicism of LES prophages.

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.