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Temperate bacteriophages as regulators of host behavior

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Bacteriophages are ubiquitous and affect most facets of life, from evolution of bacteria, through ecology and global biochemical cycling to human health. The interactions between phages and bacteria often lead to biological novelty and an important milestone in this process is the ability of phages to regulate their host's behavior. In this review article, we will focus on newly reported cases that demonstrate how temperate phages regulate bacterial gene expression and behavior in a variety of bacterial species, pathogenic and environmental. This regulation is mediated by diverse mechanisms such as transcription factors, sRNAs, DNA rearrangements, and even controlled bacterial lysis. The outcome is mutualistic relationships that enable adaptively enhanced communal phage-host fitness under specific conditions.

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Introduction

Classically, bacteriophages are viruses of bacteria, that is, selfish replicators infecting bacteria to propagate their genome. Bacteriophages, or phages, like all viruses, are obligatory parasites, dependent on their host's cellular machineries to proliferate. The abundance of these biological entities is a subject for debate but it is clear that phage virions, the phage infective particles, outnumber their bacterial hosts and it is estimated that there are around 10^{30}–10^{31} worldwide [1,2]. Still, the genetic content of phages is not well known and because of that, phages have been referred to as the 'dark matter' of the biosphere [3].

The relationship between phages and their bacterial hosts, or any obligatory parasite and its host, dictates the evolutionary dynamics of these entities. There is an arms race whereby the bacteria continuously evolve defense mechanisms against phage infections and the phages co-evolve to block, evade or subvert these mechanisms to gain an advantage in the predator–prey world [4,5]. This dynamic is a part of the 'Red Queen's race': when the host has the advantage it efficiently proliferates, outcompeting the parasite to near extinction until the parasite adapts and outcompetes its host to near extinction. These host–parasite oscillations may continue with no apparent benefit to any of the parties involved [6]. However, in some instances this dynamic promotes biological innovation and complexity, not only within each moiety as may be manifested by a novel gene or defense mechanism [4,5,7], but in the establishment of complex interactions that are mutually beneficial.

Typically, two cycles of viral reproduction are distinguished: virulent (lytic) or temperate (lysogenic). Phage–bacteria interactions during these cycles have a major role in ecology and evolution [8,9]. For example in marine environments, lytic production affects the mortality of marine microorganisms as well as their communities' structure, and is the major force behind biogeochemical cycles of nutrients, such of carbon sources [10,11]. On the other hand, lysogeny (a state of phage integration into the bacterial chromosome) provides new avenues for evolutionary and ecological dynamics between phages and their hosts [12]. Lysogeny opens a window for mutualism between the two adversaries, as now, the fitness of the prophage is tied to its host as they replicate together [13,14•]. The most obvious advantage granted to the host by integrated phages (named prophages), is superinfection exclusion, in which factors encoded in the prophage inhibit the infection of the host by other phages [15]. Another phenomenon beneficial to the host is lysogenic conversion, whereby temperate phages encode for virulence factors that enable bacteria to invade new niches and become pathogens [16•,17]. This phenomenon generally relies on the ability of phages to transfer genes from one host to another, via transduction, thus potentially increasing the fitness of the new host. Lysogeny also enables the gradual evolutionary process by which phages

go from complete selfishness (*i.e.*, lytic phages), to altruism, to complete domestication (*i.e.*, becoming cryptic phages that are unable to form infective particles) [18,19]. Indeed, the evolutionary mechanisms underlying cooptation and domestication of prophages are of great interest as they give insight into how bacteria and phages become one selectable unit [20,21]. An important milestone in phage–bacteria relationships is the ability of temperate phages to regulate host behavior, to selfish or communal benefit. There are several examples of phages playing critical roles in bacterial processes or turning into regulators of bacterial gene expression. In this review article, we will focus on newly reported cases of such bacteria–phage interactions that lead mainly to mutualistic relationships.

Phage regulation of bacterial host behavior in mammalian environments—from virulence to pathogenicity

One of the best examples of phage mediated regulation of host behavior is the regulation of biofilm formation in *Pseudomonas aeruginosa*. *P. aeruginosa* is an opportunistic Gram-negative pathogen that inhabits extremely diverse habitats, from soil to human tissues. Its capacity to establish chronic infections in humans, especially in the airways of patients with cystic fibrosis (CF), is largely dependent on the ability to form biofilms. The genome of *P. aeruginosa* Liverpool epidemic strain (LES) harbors multiple inducible prophages, and free phage particles are highly abundant in the sputa of CF patients. It is now clear that these prophages play important roles in promoting *P. aeruginosa* virulence, and that their lytic induction is critical for biofilm formation and regulation of bacterial densities at the site of infection [22]. Early reports already indicated that the typical mucoid phenotype of infection-associated bacteria (a result of massive alginate production), is in fact phage mediated [23]. An insight into the mechanism whereby temperate phages promote *P. aeruginosa* LES chronic lung infection was gained from mutagenesis studies, in which mutants exhibiting diminished virulence and reduced phage production were identified. These studies demonstrated that phage lytic induction is essential for bacterial virulence, with subsequent transcriptomic studies revealing that phage induction is associated with global changes in bacterial gene expression [24]. Experimental evolution studies in *P. aeruginosa* PAO1 suggested that prophages actively enhance parallel evolution and accumulation of mutations that are beneficial to infection, particularly in the transition to chronic infection. Mutations were identified that impair the ability of the bacteria to be motile or to regulate quorum sensing (QS) signaling, via insertional inactivation by prophage integrations of genes associated with the type IV pilus and QS. In fact, these mutations were shown to be further selected, for example, in the case of the type IV pilus, leading to the loss of motility, presumably as a result of a phage mediated mechanism that prevents superinfection by phages dependent on the type IV pilus to infect. Overall, these findings support the premise that *P. aeruginosa* prophages drive the evolution of this pathogen within the host in the course of infection [25•]. Furthermore, a remarkable interaction was observed with the temperate phage Pf (Pf4 in *P. aeruginosa* strain PAO1) that explained the need for lytic production during biofilm formation. Initially Pf phages were found to be important for biofilm development via induction of cell death and subsequent release of bacterial DNA [26]. More recently, it was discovered that the Pf particles themselves, which are long (filamentous) and negatively charged, are critical for assembly of the biofilm extracellular matrix (Figure 1b). Specifically, the phage filaments were shown to initiate formation of a highly ordered liquid crystalline matrix that enhances biofilm function and thereby, bacterial fitness and resistance to antibiotics [27••]. Pf phage production was also shown to reduce bacterial dissemination outside the lung, promote bacterial adhesion to mucin, and inhibit bacterial phagocytosis by macrophages [28]. Furthermore, it was shown to inhibit *Aspergillus fumigatus* in the airways biofilm by sequestering iron, serving as an additional weapon in the arsenal of *P. aeruginosa* against competitors [29]. In summary, it has become clear that there are multiple interactions between *P. aeruginosa* and its prophages, some of which are mutualistic and critical for *P. aeruginosa* virulence.

Another intriguing adaptation of a prophage to the lifestyle of its host was demonstrated in *Listeria monocytogenes*. *L. monocytogenes* is a food borne intracellular pathogen and the causative agent of listeriosis. During infection, the bacteria are phagocytosed by macrophage cells, though very rapidly escape the phagosomes, replicate in the cell cytosol and spread from cell to cell. Notably, bacterial phagosomal escape was shown to require genome excision of the temperate phage ϕ10403S, which is integrated into *L. monocytogenes comK* gene [30]. ComK is the master activator of the competence system, a system that enables the uptake of extracellular DNA, though in *L. monocytogenes* it was shown to promote efficient phagosomal escape [30]. During mammalian infection, ComK expression is initiated in the phagosome upon prophage excision, which leaves behind an intact *comK* gene. Remarkably, under these conditions ϕ10403S has adopted a unique behavior such that excision is not followed by lytic production, thereby avoiding bacterial lysis in the mammalian environment. The phage DNA remains as an episome and re-integrates into the *comK* gene upon bacterial replication in the host cell cytosol, essentially switching off *comK* transcription in this compartment (Figure 1a). These events reveal that although this phage is fully capable of producing infective virions (as can be shown under *in vitro* SOS conditions), during mammalian infection the lytic pathway is effectively blocked. This case exemplifies a complete adaptation of the prophage to