Prophage mediated control of higher order interactions - insights from systems approaches

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Highlights

- Prophages can impact bacterial ecology and evolution in diverse ways
- Systems biology has advanced our understanding how the effects of prophages on bacteria extend to other species and ecosystems
- Prophages contribute through cascading effects to mutualistic interactions and increased disease severity to global biogeochemical processes
- Future research should aim for integrative approaches describing how the effects of prophages on bacteria are transmitted to other levels, especially in non-model systems and in the presence of microbial communities

Abstract

Prophages, latent viral elements residing in bacterial genomes impact bacterial ecology and evolution in diverse ways. Do these prophage-mediated effects extend beyond the prophage-bacterium relationship? Here, I summarize the latest advances exploring how the impact of prophages are transmitted through multiple levels with potential impacts on ecosystem stability and functioning. The diverse effects of prophages on higher-order interactions are context-specific, ranging from contributions to global biogeochemical processes and mutualistic interactions to increased disease severity with negative impacts on ecosystem engineers and potential cascading effects for multiple species. While we have a solid understanding about the mechanisms by which prophages modulate their bacterial host at the cellular and population level, future research should take an integrative approach to quantify their effects in complex ecosystems.

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Introduction

Prophages are latent viral elements residing in bacterial genomes. They represent a specific form of a temperate phage, which upon entering a host cell can either lyse and kill the bacterium or become a prophage. Due to their high prevalence and diversity, prophages are inevitable parts of the microbial world, where they, like ruthless puppet masters, shape bacterial ecology and evolution.

Bacteria themselves, fundamental components of all global ecosystems, partake in diverse ecological interactions, not only with phages and other bacteria, but also with eukaryotic organisms. The pervasive presence of prophages and their strong impact on bacteria raises the question to what extent these viruses influence higher order interactions that go beyond the bacterium-prophage relationship? Moreover, will potential cascading effects of these interactions through multiple levels of an ecosystem impact ecosystem functioning? For example, prophages are important drivers of the viral shunt, a process in aquatic ecosystems where viral lysis of bacterial cells releases dissolved organic matter back into the water column, making it available for other organisms [1, 2]. This phenomenon significantly contributes to nutrient cycling and energy flow in aquatic ecosystems with potential upstream effects through the food-web on higher organisms and the broader ecosystem. Quantifying the impact of prophages in such multi-level processes is thus crucial for gaining a better understanding of ecosystem functioning and resilience.

However, understanding the ecological and evolutionary consequences of prophages on higher order interactions and ecosystem functioning is a difficult task because of the complex interplay between prophages, bacteria, bacterial hosts, and the environment. Therefore, we need integrative approaches that incorporate different interaction partners, integrate over different levels of organization, and generate a dynamic and evolutionary understanding by including time and space. Here, I summarized recent advances of how the impacts of prophages on bacteria are transmitted and amplified in higher-level biological interactions and highlight examples of how the cascading effects affect entire ecosystems (Figure 1).

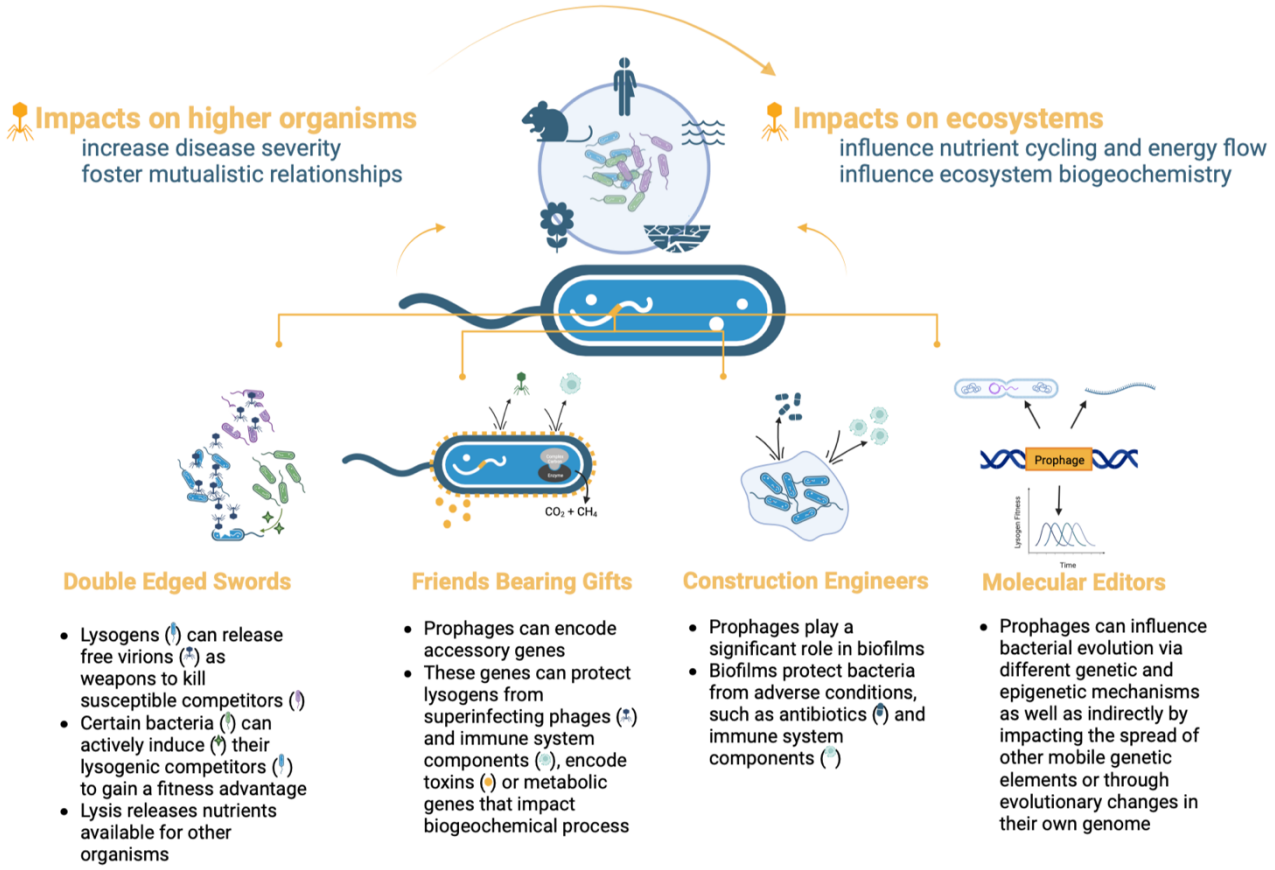

Figure 1 Four potential routes through which the impact of prophages on the fitness of their host bacterium extends beyond the prophage-bacterium relationship thereby affecting other organisms and potentially entire ecosystems. From left to right: **Double-Edged Swords**: Prophages can excise from the genome which kills the individual lysogen but released phage particles can kill surrounding phage-susceptible bacteria. Both affect the fitness of the lysogen and its surrounding bacteria and if the lysogen is a pathogen this could have far-reaching consequences for eukaryotes and, if these are key stone species, possibly for entire ecosystems. **Friends Bearing Gifts**: Prophages can encode a plethora of genes which can change the phenotype of the bacterial host and ultimately the relationship between the host bacterium and eukaryotes or in the case of certain auxiliary metabolic genes biogeochemical processes. **Construction Engineers**: Prophages play a pivotal role in biofilm formation, which in turn are important pathogenesis factors and components of mutualistic relationships between plants and bacteria. **Molecular Editors**: Prophages can influence bacterial adaptive evolution and gene expression via multiple direct and indirect ways, which can for instance in the case of pleiotropic changes influence interactions between bacteria and higher organisms.

Prophages – Double Edged Swords

Interactions among microbes are often competitive [3] and driven by similar growth needs. Prophages can switch to the lytic cycle, replicate, and lyse their host to release free phages, which can kill competing bacteria and thereby increase the fitness of the remaining lysogens, i.e., prophage carrying bacteria. Experimental [4-6] and modelling [7] approaches have repeatedly shown that the presence of one or more prophages can turn a competitive interaction into a predatory interaction characterized by phage killing. Such a beneficial effect during microbial warfare can have cascading effects. Two independent studies showed for instance that prophages, which killed surrounding phage-susceptible competitors, enhanced lysogen colonization success in a mammalian intestinal ecosystem [8, 9].

Besides directly killing competing bacteria, prophages can also indirectly influence a competitive interaction among bacteria. One example is the prophage-dependent

release of bacteriocins, that can significantly enhance lysogen fitness by killing competitors [10, 11]. A recent study revealed that temperate phages and prophages can harbour so-called biosynthetic gene clusters (BGCs) that often encode bacteriocins which can enhance lysogen fitness [11].

Prophage induction is usually lethal for the lysogen. Some bacteria exploit this "Achilles heel" of lysogens and actively induce their competitor's prophage(s) [12-14]. For instance, *Pseudomonas aeruginosa* can selectively trigger an *S. aureus* prophage through the release of phenanzine pyocyanin [14]. In this case, the authors speculate that *P. aeruginosa,* which is resistant to the released phage*,* will additionally benefit from these phages because they may kill additional phage-susceptible competitors. Similarly, during invasion of corals, the pathogen *V. coralliilyticus* induces the prophages of competing non-toxigenic Vibrio sp. and other coral symbionts by releasing H_2O_2. A genome-wide-association study, revealed that the LodAB operon, which mediates H_2O_2 release, is widely distributed among bacteria and possibly constitutes an important feature facilitating invasion of healthy microbiomes by pathogens [13]. This highlights that a prophage that negatively affects the fitness of an important ecosystem engineer such as corals, may have far-reaching consequences across entire ecosystems with cascading effects on plants, fishes, and invertebrates. However, more studies, that consider entire species networks are needed to quantify the overall effect of prophage induction and subsequent lysis on ecosystem stability and resilience.

Prophages – Friends bearing gifts

During lysogeny, bacteria and prophage fitness is tightly intertwined, resembling a mutualistic relationship. Prophages can increase lysogen fitness through lysogenic conversion, i.e., by carrying non-essential genes whose expression modifies the bacterial phenotype [15]. The first described example of lysogenic conversion that turned a harmless bacterium into a deadly pathogen is the prophage born origin of the cholera disease [16]. Since then, many other prophage-encoded virulence genes, which are often species-specific [17], but also non-virulence genes with selective benefits, have been discovered. These include genes, that protect bacteria from superinfection by other phages [18] and environmental stress [19], increase serum resistance [20] or enable the host bacterium to access new metabolic resources [21-24]. Consequently, the expression of these genes and the resulting phenotype changes can influence existing ecological interactions, that involve the lysogen, in diverse ways.

One example are auxiliary metabolic genes (AMGs), which can alter the metabolism of their hosts and influence ecosystem biogeochemistry (see [25] for a review). While the rise of metagenomic and viromic data is revealing an ever-increasing number of putative phage-encoded AMGs, knowledge on their function often remains elusive, because we lack sufficient phage-host systems to confirm their predicted function [22]. Alternative integrative approaches to circumvent the lack of suitable systems include, amongst others,

comparisons of prophage to host gene ratios which revealed the importance of prophages in sulphur and thiosulphate oxidation [23] or the use of heterologous expression systems which identified phage-encoded carbohydrate-active enzymes that play an important role in the break-down of complex carbon to CH_4 and CO_2 [24]. However, without culturable phage-host systems it remains challenging to estimate the real contribution of these AMG-carrying phages to global biogeochemical processes and their effects on ecosystems [22].

Bacteria that coexist with higher organisms are constantly exposed to their immune system. Prophages have acquired multiple mechanisms to protect their host from this threat. This includes their ability to inhibit local inflammatory and immune reactions, in particular phagocytosis (for a review see [26]). One of the most recently discovered examples are ankyphages, that encode and express the immunomodulatory ankyrin protein (ANKp). ANKp, which reduces phagocytosis rates, fosters the mutualistic relationship between sponges and ANKp-lysogens, and possibly many other mutualistic host-microbe relationships [27]. However, in the case of pathogens, such prophage-encoded immune-evasion genes can increase disease severity with negative consequences for the eukaryotic host.

Another hazard prophages can protect their host from, are superinfecting phages. Indeed, many prophages carry superinfection exclusion (SIE) proteins that can for instance modify cell surface structures preventing phage attachment. If accompanied by a loss of function, such modifications can however, negatively impact lysogen fitness [28, 29]. Thus, prophage-encoded SIE proteins could change the outcome of a competitive interaction among bacteria or the colonization success of a pathogen for the worse. Only a few studies have investigated the cascading effects of SIE proteins. One example is a systematic study on 30 closely related prophages of *Pseudomonas aeruginosa*, which found no additional fitness cost of SIE for the lysogen [30]. This is however in stark contrast to SIE mediated by filamentous phages. The massive amplification and subsequent release of viral particles through phage-encoded proteins inserted into the bacterial cell membrane significantly reduces lysogen growth [31-33]. Thus, despite their ability to protect their host from super infecting phages, their high fitness cost renders filamentous phages more susceptible to extinction if phage-resistant cell surface mutants emerge [33]. While there are many described individual examples of prophage-encoded SIE proteins, their ecological and evolutionary impact on higher order interactions remains unclear. That is because we lack a detailed understanding of their net fitness effect for lysogens and of how widespread these different SIE mechanisms are. Integrating large scale genome mining to uncover the nature and distribution of SIE across different microbial ecosystems with modelling that predicts their impact on higher order interactions represents an exciting possibility for future research.

Prophages – versatile construction engineers

Prophages are pivotal to the formation and maintenance of biofilms [34-38]. Within biofilms, bacteria are protected from adverse conditions, including for instance antibiotics [39] and immune system components [40]. This makes biofilms an important pathogenesis factor [34, 38] and exemplifies how the impact of prophages can manifest as cascading consequences across multiple species.

However, biofilms are also important components of many terrestrial and aquatic ecosystems where they form the basis of food-webs and maintain nutrient cycling and bioremediation; for a review see [41]. Thus, in contrast to clinical biofilms, prophages that contribute to the formation of ecological biofilms can support mutualistic interactions. One example of this phenomenon can be observed in the symbiotic association between *Phaeobacter inhibens* and microalgae, which relies on the lysogenic state of *P. inhibens* to form biofilms on the surface of the algae and is likely attributable to prophage-encoded genes in *P. inhibens* [42].

Given that biofilms exhibit a higher level of productivity compared to their planktonic counterparts, they are of significant relevance to ecosystem functioning. While we have a detailed understanding about the mechanisms by which prophages influence biofilm formation, it is difficult to quantify the functional significance of prophages for the biofilm and the implications on subsequent ecological interactions. That is because we lack quantitative data and adequate theoretical models that allow us to reveal the temporal dynamics of prophage-biofilm interactions in different environments.

Moreover, there is growing evidence that the biofilm lifestyle may be more mutagenic than the planktonic one and that virulence and antibiotic resistance genes are more efficiently distributed via horizontal gene transfer (HGT) within biofilms [43]. If the functioning of these biofilms depends on prophages, they may indirectly influence the evolution of multidrug resistant pathogens and worsen the outcome for the eukaryote.

Prophages – molecular editors

Like all mobile genetic elements (MGEs), temperate phages play a significant role in shaping the molecular landscape of bacteria and, therefore, impacting bacterial evolution. Commonly known is their ability to act as vectors for HGT, either via transduction or lysogenic conversion, allowing bacteria to acquire new genes and traits that can impact higher order interactions. However, recent advances identified a variety of additional genetic and epigenetic mechanisms through which prophages impact bacterial evolution.

Prophage integration can accelerate adaptive evolution either directly through the acquisition of adaptive prophage-encoded genes, which can occur at a faster rate than de novo mutations [44] or indirectly by integrating into protein coding sequences which can increase the supply of beneficial mutations [45]. Both mechanisms are beneficial for bacterial fitness and can in the case of colonizers foster host-symbiont relationships [44]

or in the case of pathogens facilitate the transition from acute to chronic infections likely increasing disease severity [45].

Prophages can also regulate the expression of bacterial genes through various mechanisms. For example, prophages can reverse the disruption of host genes through controlled excision, acting as genetic switches [46, 47]. This process, defined as active lysogeny [46], can regulate phagosomal escape and virulence in the intracellular pathogen *Listeria monocytogenes* [47], and facilitate rapid, parallel adaptation in *P. aeruginosa*, enabling the establishment of chronic infections [48]. Prophage integration can regulate the expression of bacterial genes by controlling the transcription of nearby genes [49] or by causing changes in the bacterial chromatin structure [50], which likely affects the accessibility of the genetic material to the transcriptional machinery. This can result in changes in the expression of genes involved in various cellular processes such as metabolism, growth, and virulence. Thus, by altering the expression of genes involved for instance in stress response and adaptation, prophages can also influence the adaptive response of bacteria to environmental changes.

Environmental change in turn can significantly influence the shape and trajectory of bacteria-prophage co-evolution. For instance, environments that reduce bacterial growth rate can inhibit phage infections (for a review see [51]), which can slow down phage resistance evolution and prolong phage epidemics [52]. Moreover, environmental conditions that influence the balance between lysis and lysogeny drive the emergence of phage resistance mutations and subsequent prophage loss, which might explain why prophage prevalence varies across environments [53]. Thus, changing environments provide an additional way by which the impact of prophages can spread quickly across microbial populations and beyond. This highlights the importance of considering environmental factors in future studies in this field.

Prophages can also indirectly influence bacterial evolution. For instance, by driving bacterial counteradaptation, pleiotropic effects of evolved phage resistance can occur [33, 54]. Here, molecular, or epigenetic alterations of cell appendices such as type IV pili or O antigen structures that prevent phage re-infections can create an evolutionary trade-off between phage resistance and bacterial fitness [33, 54, 55]. By acting as an additional death rate for carriers of other MGEs, such as plasmids, prophages can constrain the horizontal spread of these MGEs, which can slow down plasmid mediated adaption [56]. Through evolutionary changes in their own genome, e.g., changes in genes affecting phage release rate, which positively correlates with bacterial virulence, prophages can indirectly shape bacterial phenotypes [54].

Despite a growing understanding of the mechanisms by which prophages influence bacterial evolution in controlled laboratory settings, there is still a significant knowledge gap regarding the evolution of prophages and bacteria in natural environments, and how this influences the evolution of higher organisms and the cascading effects thereof. Considering that bacteria co-evolve with higher organisms, a pertinent question for future

research is to investigate the extent to which prophages shape these co-evolutionary dynamics, and through what mechanisms.

Conclusion

Holistic and multi-scale studies have provided ample evidence that prophages significantly affect bacterial ecology and evolution, which translates via cascading effects to higher organisms and ecosystem functioning. Although we have a solid understanding of the mechanistic ways in which prophages influence their bacterial hosts at the cellular and population level, there is still much to investigate to fully understand the impact of prophages across multiple levels. Two of the key areas where more research is needed include:

- Understanding of prophage function and maintenance in different ecological niches. We mainly study prophages in the context of human and animal hosts, and less in soil, water, and plant associated microbes where their role and impact may be different
- We are only beginning to understand the role of prophages in host-microbiome interactions. However, a recent synthesis suggests that the context of the bacterial community is important for interactions between virulent phages and bacteria [57], and therefore *in vitro* observations may not always hold true *in vivo* [58]. Thus, more research is needed to unravel the complex interplay between prophages and the host's microbiome and to elucidate how the microbiome, in turn, shapes the host's response to prophages.

Thus, to decipher the magnitude of prophages on higher order interactions and complex ecological systems, future work requires multi-disciplinary approaches encompassing a combination of computational and experimental techniques with a focus on non-model systems. This includes genomic and transcriptomic analyses of phage-containing microbiomes to reveal the genetic make-up and expression patterns of prophages in these systems. Additional functional assays, such as measuring lytic vs lysogenic activity can reveal the impact of the dynamics of a prophage's lifecycle while advanced imaging techniques can provide mechanistic insights at the cellular or sub-cellular level. Integrating these data into predictive models that simulate interactions among prophages, bacteria and higher order interaction partners under different conditions will enable us to gain a deeper understanding of how prophages influence complex ecological systems. Such an understanding is crucial, given the global importance of bacteria for both our existence and the fundamental ecological processes that govern our planet.

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