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When bacteria are phage playgrounds: interactions between viruses, cells and mobile genetic elements

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Abstract

Studies of viral adaptation have focused on the selective pressures imposed by hosts. However, there is increasing evidence that interactions between viruses, cells, and other mobile genetic elements (MGEs) are determinant to the success of infections. These interactions are often associated with antagonism and competition, but sometimes involve cooperation or parasitism. They involve mechanism of defense or genetic regulation that evolve by genetic exchanges followed by processes of co-option and diversification producing multi-layered networks of interactions. Gene exchanges thus facilitate the emergence of cross-talk between elements that co-inhabit the same bacterium. This creates opportunities for the exploitation of phages by other MGEs in the cell and for their manipulation by competing bacteria.

Introduction

Bacteriophages (phages) are viruses of bacteria. Their diverse virulence strategies shape the duration, impact, and complexity of the interactions they have with the bacterial host. Virulent phages follow infection cycles that end with cell lysis and liberation of progeny. Occasionally, for reasons not fully understood, these phages can remain in cells in a non-replicative state, called pseudolysogeny, before the onset of the lytic cycle. Temperate phages can either follow the same type of lytic cycle or integrate the genome as prophages, in which case they are effectively part of the bacterial genome. They are found in at least half of the bacterial genomes and can account for a substantial part of bacterial gene repertoires [1]. Other phages have peculiar alternative lifecycles. For example, filamentous phages continuously produce viral particles that they export to the outside of the living cell (chronic life cycle without cell lysis) [2].

Phages are very abundant predators that influence the dynamics of bacterial populations and provide the host with adaptive traits. The type and duration of the interactions between viruses and the cell are thus determinant for the success of phage infections and for bacterial survival. These interactions vary with the type of phage and the host genetic background. Some phages develop multiple different genetic programs (temperate phages), some function continuously in living cells (filamentous), and others quickly kill their hosts (virulent). The costs and opportunities provided by the lifecycle of the phage affect the evolution of these interactions. For example, one expects cells to systematically evolve defenses against virulent phages because they tend to be deadly. In contrast, temperate phages may kill the host upon inducing the lytic cycle or integrate its genome and eventually increase its fitness by providing it with novel phenotypes (lysogenic conversion). Therefore, interactions between temperate phages and the bacterial host may be more subtle and varied. Beyond these pairwise phage-bacterium interactions one must consider that phages are not alone within cells. Bacterial genomes have many other mobile genetic elements (MGEs) with whom phages may establish relations of competition, antagonism, or parasitism. In this context, cells can be regarded as playgrounds for interactions between MGEs that impact the outcome of phage infections and the rate of horizontal gene transfer between bacteria [3]. Here, we focus on three types of molecular interactions faced by phages upon injecting their DNA in host cells: defense systems, regulatory interactions, and recombination. These interactions have typically been studied independently, but there is increasing evidence that their interplay can have unexpected outcomes.

Defense systems: conflict and cooperation

All of life's evolution is a history of incessant conflict between hosts and parasites. Bacteria have evolved numerous immune mechanisms, both innate and adaptive, to defend themselves from phages [4,5]. These mechanisms, whose complexity and diversity are quickly being unraveled, target each step of the phage reproduction cycle, from blocking its attachment to the cell, to cleaving phage nucleic acids, to spurring metabolic arrest or even cell suicide [6]. They either protect cells from phage infection or stop epidemic waves by inducing cell death [7]. Surprisingly, recent results suggest that many, or even most, such so-called bacterial defenses rely on MGEs.

The rapid evolution of the antagonistic interaction between phages and bacteria drives rapid change of defense and anti-defense systems. This may occur by sequence evolution, *e.g.* change of CRISPR spacers to match novel phages, but in most defense systems it requires frequent acquisition of novel systems by horizontal gene transfer, which are usually co-transferred by

MGEs or encoded in the MGEs themselves. In marine *Vibrio*, MGEs encode most of the cell defense systems. The turnover of MGEs in genomes, and their anti-phage systems, explains much of the differences in susceptibility of closely related strains to different phages [8,9]. MGEs encode many anti-phage systems presumably because when phages are killing the bacteria, they are also eliminating the MGEs. By defending bacterial cells, most MGEs are defending themselves.

Many of the anti-phage systems are encoded in temperate phages, that, as prophages, defend bacteria from other phages [8,10-14]. These phage-encoded bacterial defense systems have interesting characteristics. For example, their CRISPR-Cas systems are extremely compact [15]. Sometimes they even lack *cas* genes and consist only of CRISPR mini-arrays that use the *cas* machinery from the host [16]. Such simple systems are implicated in competition between phages [17]. They fit the 'guns for hire' model whereupon phages acquire anti-phage systems through recombination with their bacterial hosts [18], or with other MGEs, thus blurring the boundaries between defense and counter-defense systems. This frequent transfer and co-option of defense and anti-defense systems highlights the importance of gene flow, which we will expand on below, in the evolution of phage-bacteria interactions.

Phages have their own parasites. Phage satellites exploit "helper" phages by hijacking their viral particles to ensure their own horizontal transfer [19]. Recent studies suggest they are very abundant [20,21]. Certain satellites completely block phage reproduction, e.g. the PLE satellite of *Vibrio cholerae* aborts the production of ICP1 phages [22]. Its activity still kills the cell, but benefits the bacterial population by preventing the epidemic spread of the phage. In other cases, the ecological interactions between phages and their satellites are more subtle. The P4-like satellites of enterobacteria have only a small impact on the "helper" phage reproduction and encode numerous anti-phage systems that protect them, the "helper" phage, and the cell from other phages [14]. Hence, satellites are hyper-parasites (parasites of parasites) that can defend bacteria from the "helper" phage or defend the cell and the "helper" phage from other phages.

Phages have evolved numerous tools to protect themselves from the cell defense systems. These anti-defense strategies may depend on cooperative interactions with other phages [23]. For example, the presence of multiple infecting phages producing anti-CRISPR proteins improves the chances of neutralization of the host CRISPR-Cas activity by increasing the number of such proteins in the cell cytoplasm [24,25]. These anti-anti-phage proteins are thus a sort of intracellular public good. As a result, they can be used, and even exploited, by other phages or MGEs that benefit from the associated immunosuppression without paying its cost [26]. This opens the opportunity for dynamic, complex, and multi-layered social interactions within the cell.

The key role of MGEs in bacteria-phage interactions raises intriguing questions around the real roles of defense systems in cells and whether selection for their presence is always in the host's interest [27]. For example, the presence of anti-phage systems in integrative conjugative elements of *Vibrio cholerae* decreases the likelihood of acquisition of phages carrying virulence factors [28]. For a better understanding of the *raison d'être* of these defense systems, one thus needs to better characterize both the mechanisms of defense systems and the costs and benefits of the MGEs encoding them. It's also important to note that once the phage passes cell defenses, its fate will still depend heavily on its regulatory interactions with other MGEs and with the host. We will go through these below.

Gene regulation for the control of ecological associations

The regulation of gene expression has a key role in phage-bacteria interactions. Phages must regulate the expression of their own genes, which may be particularly complex for temperate phages that must manage different programs of gene expression (lysis/lysogeny). But phages can also regulate host functions using their DNA, RNA, or regulatory proteins. Temperate phages may act as genetic switches by interrupting/re-establishing a gene when integrating the chromosome, a process termed "active lysogeny". This strategy may co-evolve with the host to regulate gene expression in specific niches, e.g. intracellular survival [29]. Phage-integration was shown to re-program *Escherichia coli*'s anaerobic respiratory system through transcription of neighboring chromosomal genes initiated from a phage promotor [30]. Phage encoded small RNAs can also contribute to phage-bacteria cross-regulation, e.g. by affecting the regulation of bacterial core genome transcripts [31]. Recently, it was shown that prophage-encoded quorum sensing (QS) regulators allow phages to eavesdrop on bacterial communication systems to sense host cell-densities and eventually switch to the lytic cycle [32]. Phage protein regulators may also activate expression of virulence genes in the bacterial host [33], or down-regulate its SOS response [34].

Bacteria can also regulate the expression of phage genes. The transcription termination factor Rho silences phage genes by acting as a transcription termination factor. In *E. coli* it maintains prophages in that state by preventing their excision from the chromosome [35]. Bacteria also encode small nucleoid-associated proteins that bind and downregulate the expression of the AT-rich phage genes. These proteins are collectively referred to as xenogeneic silencers (XS) and their mode of action contrasts to that of other bacterial defense mechanisms in that it is not destructive of its targets. The ability to silence and stabilize prophages gives the cell an opportunity to benefit from lysogenic conversion (reviewed in [36]). The impact of XSs may span multiple regulatory levels. The *Shewanella oneidensis* host-encoded H-NS protein represses or derepresses prophage excision depending on post-translational modifications. Under low-temperature, this XS favors prophage excision which ultimately results in the expression of cold-shock survival genes [37]. When temperature rises, the prophage re-integrates the chromosome. In this case, the host regulates the excision of a prophage that works for the host as a genetic switch.

Phages and other MGEs can also encode XS**.** The first of these were initially identified in plasmids, where they stabilize the presence of plasmids by decreasing their fitness cost [38]. XS homologs were found in the genomes of actinophages [36] and through metagenomics in phages infecting other bacterial clades [39]. For instance, the XS encoded in a cryptic prophage of *Corynebacterium glutamicum* silences various AT-rich host genes (beyond regulating the prophage itself). The interference caused by this XS activity could be counter-acted by foreign XSs (H-NS from *E. coli*) and by truncated variants of the XS itself [40]. This means that truncated variants of XSs can act as anti-XS proteins [41]. Such proteins could easily evolve from existing XS, allowing phages to interfere with the XS activity of the host. Other proteins used by phages to control bacterial XS are not homologous to XS and use alternative mechanisms [42]. Overall, this paves the way for competition for the genetic control of phage-bacteria interactions based on XS and anti-XS functions, which has striking parallels with that of defense and counter-defense systems.

Of all the intracellular interactions between MGEs, those of resident prophages are amongst the most vital for infecting phages. The presence of prophages in bacteria may protect the ensemble from virulent phages because of the defense systems they encode (see above). But prophages

also interact with incoming phages in other ways, which are sometimes poorly understood. For example, prophages may provide protection from pseudolysogeny of virulent phages [43]. They may also protect the cell from closely related temperate phages by repressing them. The lytic cycle of prophages is usually actively repressed to avoid its untimely induction. The presence of these repressors in the cell precludes super-infection by the same phage, but can also repress incoming phages with similar regulatory sequences [44]. Such mechanisms have been poorly studied and their relevance to control a broad range of phages is yet unclear. The study of repressor-mediated immunity in a panel of *Pseudomonas* strains revealed that this mechanism was responsible for a small number of cases of resistance to different temperate phages [10]. The relatively minor role of super-infection exclusion on cell defense may be due to the rapid evolution of regulatory systems that could rapidly make native repressors incompatible with distinct incoming phages. Nevertheless, repression of incoming phages is a relevant component of prophage-encoded mechanisms that protect the bacterial host (and thus the prophages themselves). Albeit very different from the double stranded DNA phages that are the focus of this review, filamentous phages and microvirus also encode multiple superinfection exclusion systems, which influence the infection dynamics of unrelated co-infecting viruses [45,46].

Since some strains have up to 20 prophages, there is a potential for complex regulatory interactions between resident prophages. It has been shown that the presence of two phages in the cell has deleterious consequences for their productivity [47], even if the mechanisms are poorly understood. The complexity of the underlying interactions is illustrated by two pairs of *Salmonella enterica* prophages [48]. Prophages Gifsy-1 and Gifsy-3 do not repress each other, but their repressors can be cross-targeted by the other prophages' anti-repressor proteins. Hence, de-repression of one leads to the induction of the other. The other pair shows a more complex mechanism. The repressor of prophage Gifsy-2 is sensitive to the anti-repressor of Fels-1, but Fels-1 induction does not depend on its own anti-repressor. This suggests that Fels-1 anti-repressor is only used by the prophage to manipulate other prophages. Another striking example of cross-regulation was identified in *Listeria monocytogenes*, where a prophage encoded gene has two functions resulting from two different protein domains. One of the domains regulates the expression of the prophage [34]. The other domain downregulates the SOS response by inhibiting RecA. Since many MGEs are induced by the SOS response, this dual-function regulator can regulate the induction of the phage and of many other elements in the cell, including other prophages.

These examples show that the presence of regulators in bacteria, phages and other MGEs generates a complex network of regulatory interactions that shapes the presence and activity of phages in their bacterial hosts. This network keeps changing because of the rapid turnover of phages and other MGEs in cells and of genetic exchanges between them.

Recombination drives gene flow

Many phages have mosaic genomes, i.e. interspersed regions with high similarity to distinct phages, that are the result of multiple independent events of recombination with other phages. Because the phage genome is isolated from other genomes within the viral particle, these recombination events must result from interactions taking place within the bacterial cell. Recombination is expected to be more frequent between temperate phages than between virulent phages, because the former have very long periods of co-existence as prophages providing opportunity for recombination with infecting phages or other resident prophages [49]. Recombination between phages is facilitated by their modular genome organization, where closely related functions tend to be encoded together, allowing the exchange of complete

functional modules in a single event [50]. For example, lambdoid phages have similar genetic maps and recombination between these phages, which are sometimes highly divergent, can provide viable hybrids.

A few recent studies attempted to quantify genetic flux between phages. The analysis of pairs of phages in terms of nucleotide distance versus gene content similarity shows two modes of phage evolution that are consistent with high or low gene flux [51]. Recombination between temperate phages is frequent and associated with high gene flow. Accordingly, evolution experiments in the lab uncovered recombinants between prophages of *Bacillus subtilis* that resulted in the generation of virulent phages [52]. But recombination between virulent phages is not rare. The follow-up of a lineage of virulent *Siphoviridae* for over 29 years revealed a constant rate of five recombination events per year that caused ca. 24 times more changes to genomes than point mutations [53]. Hence, even virulent phages interact frequently enough within cells to exchange DNA many times in a relatively short period of time. Some of these genetic exchanges may be caused by defense systems that target the DNA sequences of phages. For example, restriction-modification systems produce recombinogenic linear double stranded DNA that might promote recombination.

On the other edge of the evolutionary scale, some very dissimilar phages, including pairs of virulent and temperate, have almost identical genes, a hallmark of gene flow [54]. Such processes presumably take advantage of the peculiar molecular mechanisms of recombination encoded by phages that allow exchanges between more dissimilar DNA sequences than is possible by bacterial homologous recombination [55]. Accordingly, recombination is more frequently observed in phages encoding such mechanisms [54]. Since other MGEs, e.g. ICEs of *Vibrio* [56], encode similar recombinases, it is tempting to speculate that the recombination mechanisms themselves can be transmitted between phages and other MGEs. Recombination between unrelated phages have consequences for the transfer of genes across phage families or between phages infecting distant bacterial taxa. Even if virulent phages recombine more rarely than temperate phages, they can recombine with phages infecting more distant bacterial clades [54]. This suggests that virulent phages have a broader host range and could shuttle genes between temperate phages infecting distantly related bacteria.

The genes transferred between phages include defense systems, anti-defense systems, regulators, and recombinases, which are often like those of bacteria or other MGEs [14,54]. Other functions reported to be transferred to and from phages include integrases [57] and tRNAs to compensate differences in codon usage [58,59]. This suggests that genetic exchanges between phages, hosts, and other MGEs are common. Such functions find a novel context in the recipient genome and may end up providing novel phenotypes and even novel functions [60]. Unfortunately, there has been little work on the systematic identification of these exchanges.

Genetic exchanges between phages and other MGEs could also be at the origin of new types of MGEs such as phage-plasmids or phage satellites. Phage-Plasmids (P-Ps) are temperate phages that replicate autonomously as plasmids within bacterial cells. A recent systematic screening for these elements in bacterial genomes found that ˜6% of all plasmids and phages are P-Ps [61]. These P-Ps form large, presumably ancient, families of mobile elements that may have originated from events of recombination between phages and plasmids or may represent vestiges of a period when such elements were less distinct than they tend to be today. Interestingly, while phages have few transposable elements and antibiotic resistance genes, P-Ps have many of these, sometimes grouped in integrons, just like plasmids [62]. Hence, the

similarities of P-Ps with both phages and plasmids may allow them to drive gene flow between the two types of elements.

Phage-inducible chromosomal islands (PICI) are phage satellites found in Proteobacteria and Firmicutes that encode DNA packaging machineries homologous to those of phages [63]. While there is little information on their evolution, such genes could have been acquired from phages a long time ago, when PICI emerged. In contrast, other phage satellites such as P4 [21] or the PLE of *Vibrio* [64] have few phage homologs. Yet, even some of the hijacking mechanisms of the latter may have been acquired from phages. For example, the *capR* gene of PLE encodes a DNA-binding protein thought to be derived from a phage homing endonuclease. This protein represses the "helper" phage capsid morphogenesis operon [64]. Hence, a satellite obtained by genetic exchange (recombination) a gene of the "helper" phage that it uses to control the genetic expression of the latter, thereby defending the cell from this virulent phage. This nicely sums up the interplay between the three different types of interactions described in this review.

Conclusion and perspectives

The last few years revealed numerous cases of interactions between phages and the host or other MGEs in the cell. These discoveries are ongoing at an ever faster pace [65]. While it is tempting to regard defense systems as primarily anti-phage systems, this may not always be the case. For example, the *Vibrio cholerae* El Tor strain responsible for the seventh cholera pandemic lacks plasmids because it encodes two defense systems that cooperate to eliminate small multi-copy plasmids [66]. One of the two systems also targets phages and we are tempted to speculate that phages may in this case be collateral victims of a bacteria-plasmid antagonistic interaction. Likewise, some of the regulatory interactions acting upon phages in cells may also be side effects of interactions between the large network of MGEs present in bacterial genomes.

As we saw above, phages may develop mechanisms to evade deleterious interactions with the cell or other MGEs. Some phages encode proteinaceous nucleus-like compartments that encapsulate processes such as DNA replication, recombination, and transcription [67], and protect its DNA from certain anti-phage systems [68,69]. These compartments may protect the phage from regulatory interactions with the host, but could also decrease its ability to engage in genetic exchanges.

The existence of numerous interactions between phage and MGEs opens the possibility of their manipulation by other biological agents. We discussed above how satellites can manipulate phages. Interestingly, prophages can also be manipulated by other bacteria. *Streptococcus pneumoniae* displaces *Staphylococcus aureus* lysogens from the nasopharynx by producing peroxide hydrogen that kills the latter by inducing the lytic cycle of its prophages [70]. Given that prophages are "molecular timebombs", their exploitation by competitor bacteria might be common. This could occur by manipulation of the quorum-sensing mechanisms that prophages use to sense conditions favorable for prophage induction [71,72]. It is tempting to speculate that bacteria could induce the prophages of competitors by interfering with such signals. This could result in alleviation of prophage repression, induction of the lytic cycle, and cell death.

Phage-bacteria antagonistic interactions have led to the continuous evolution of strategies by which one partner aims at inactivating or controlling the other. Since both partners evolve very fast, this results in rapid turnover of the gene repertoires associated with these interactions. When interactions with the host and its MGEs are systematically negative, as is the case for virulent phages, one may expect strong selection for inactivating defense systems. But when

interactions oscillate between parasitism and symbiosis, as in temperate phages, the destruction of the partner may be disadvantageous. This has led to the development of mechanisms that aim at controlling gene expression of the other partner instead of destroying it. In both situations, the molecular mechanisms used by phages and bacteria are often homologous, which is the result of frequent recombination between them.

Hence, within bacterial cells, networks of interactions between bacteria, phages, and other mobile genetic elements keep evolving and shaping their rates of adaptation.

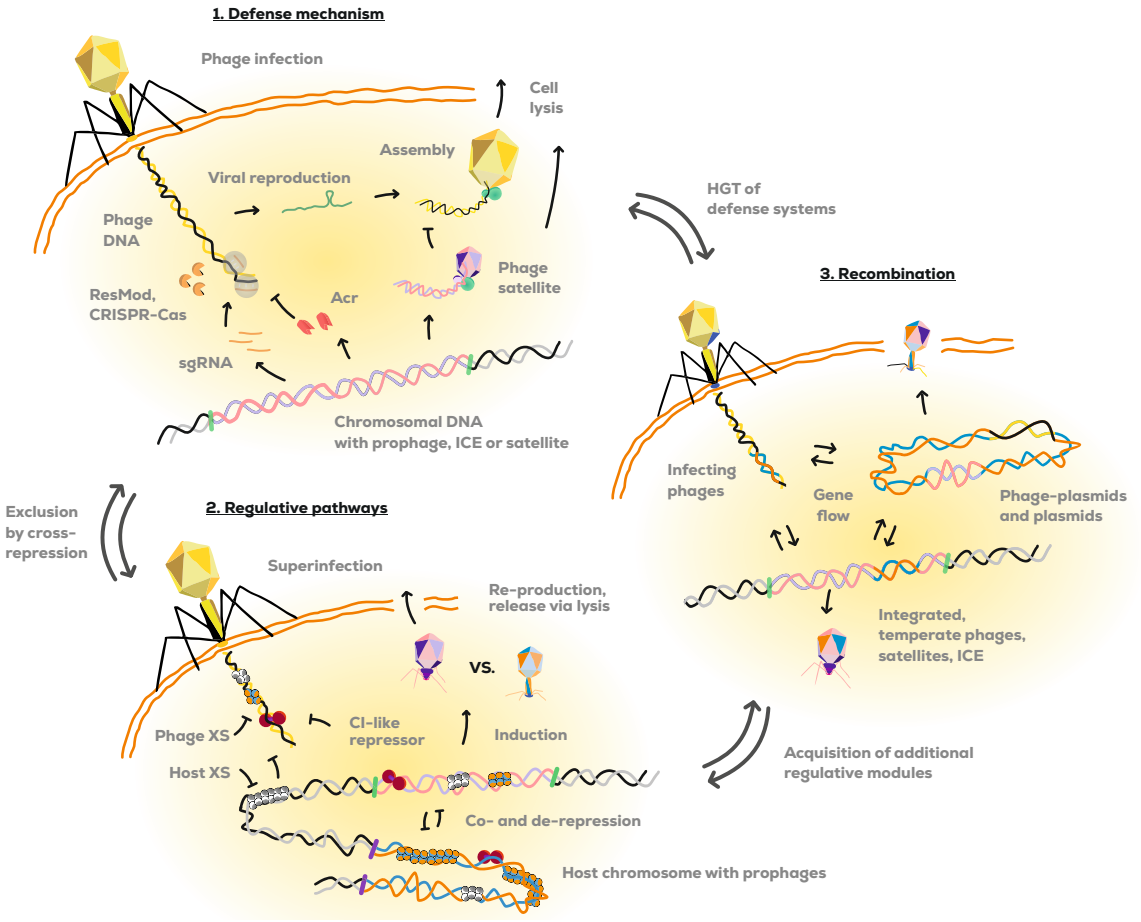


Figure 1: Genetic interactions of phages with other MGEs inside the host.
1. Bacteria encode defense mechanisms against phages (such as CRISPR-Cas or restriction modifications systems) to prevent viral reproduction. MGEs can encode anti-CRISPR proteins (Acr) that inactivate the hosts Cas proteins promoting the fitness of themselves and the incoming phage. Phage satellites usually hijack the assembly/packaging machinery of the helper phage and can block the production of new infectious viruses. **2**. Temperate phages have regulatory pathways to downregulate the expression of lytic genes and maintain lysogeny. They can co-repress related phages and prophages, preventing superinfection. XS proteins of different types, phage and host-encoded, bind and downregulate expression of phage AT-rich DNA. Incompatible XS functions may cause interference (de-repression), whereas similar XS can co-regulate plasmids, prophages, and phages. **3.** Defense systems, regulatory pathways and other genes can be exchanged by recombination between phages, plasmids, phage-plasmids, prophages, ICE, or satellites.

Questions for further research

How many of the unknown function genes are dedicated to interactions between phages and MGEs?
What are the networks of interactions of phages in cells?
How much of phage host range is defined by intracellular interactions?
Which processes favor gene exchanges between phages, the cellular genome and other MGEs?
How do phage interactions within a bacterium contribute to the fate of infection?
Is there a regulation of cell defenses?
Can the regulatory cross-talk within cells be systematically subverted by phages and competitor bacteria?

Highlights

- Phages and bacteria have hyper-variable defense and counter-defense systems
- Extensive regulatory cross-talk between phages, MGEs and the bacterial host.
- Defenses and regulators are frequently exchanged by recombination.
- Interactions between phages and bacteria are affected by other MGEs.
- Phages may sometimes be victims of collateral damage of other interactions.

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References

1. Touchon M, Bernheim A, Rocha EP: **Genetic and life-history traits associated with the distribution of prophages in bacteria**. *ISME J* 2016, **10**:2744–2754.
2. Hay ID, Lithgow T: **Filamentous phages: masters of a microbial sharing economy**. *EMBO Rep* 2019, **20**:e47427.
3. Haudiquet M, de Sousa JM, Touchon M, Rocha E: **Selfish, promiscuous, and sometimes useful: how mobile genetic elements drive horizontal gene transfer in microbial populations**. *EcoEvoRxiv* 2021, **10.32942/osf.io/7t2jh**.
4. Doron S, Melamed S, Ofir G, Leavitt A, Lopatina A, Keren M, Amitai G, Sorek R: **Systematic discovery of antiphage defense systems in the microbial pangenome**. *Science* 2018, **359**:eaar4120.
5. Tesson F, Hervé A, Mordret E, Touchon M, d'Humières C, Cury J, Bernheim A: **Systematic and quantitative view of the antiviral arsenal of prokaryotes**. *Nature communications* 2022, **13**:1-10.
6. Egido JE, Costa AR, Aparicio-Maldonado C, Haas P-J, Brouns SJ: **Mechanisms and clinical importance of bacteriophage resistance**. *FEMS microbiology reviews* 2022, **46**:fuab048.
7. Lopatina A, Tal N, Sorek R: **Abortive infection: bacterial suicide as an antiviral immune strategy**. *Annual Review of Virology* 2020, **7**:371-384.
8. Hussain FA, Dubert J, Elsherbini J, Murphy M, VanInsberghe D, Arevalo P, Kauffman K, Rodino-Janeiro BK, Gavin H, Gomez A, et al.: **Rapid evolutionary turnover of mobile genetic elements drives bacterial resistance to phages**. *Science* 2021, **374**:488-492.
9. Piel D, Bruto M, Labreuche Y, Blanquart F, Chenivesse S, Lepanse S, James A, Cruz RB, Dubert J, Petton B: **Genetic determinism of phage-bacteria coevolution in natural populations**. *bioRxiv* 2021.
10. Bondy-Denomy J, Qian J, Westra ER, Buckling A, Guttman DS, Davidson AR, Maxwell KL: **Prophages mediate defense against phage infection through diverse mechanisms**. *ISME J* 2016, **10**:2854-2866.
11. Dedrick RM, Jacobs-Sera D, Bustamante CA, Garlena RA, Mavrich TN, Pope WH, Reyes JC, Russell DA, Adair T, Alvey R, et al.: **Prophage-mediated defence against viral attack and viral counter-defence**. *Nat Microbiol* 2017, **2**:16251.
12. Piel D, Bruto M, Labreuche Y, Blanquart F, Goudenège D, Barcia-Cruz R, Chenivesse S, Le Panse S, James A, Dubert J, et al.: **Phage-host coevolution in natural populations**. *Nat Microbiol* 2022, **7**:s41564-41022-01157-41561.
13. Owen SV, Wenner N, Dulberger CL, Rodwell EV, Bowers-Barnard A, Quinones-Olvera N, Rigden DJ, Rubin EJ, Garner EC, Baym M: **Prophages encode phage-defense systems with cognate self-immunity**. *Cell Host & Microbe* 2021, **29**:1603-1605.
14. Rousset F, Depardieu F, Miele S, Dowding J, Laval AL, Lieberman E, Garry D, Rocha EPC, Bernheim A, Bikard D: **Phages and their satellites encode hotspots of antiviral systems**. *Cell Host Microbe* 2022, **30**:740-753.e745.
15. Pausch P, Al-Shayeb B, Bisom-Rapp E, Tsuchida CA, Li Z, Cress BF, Knott GJ, Jacobsen SE, Banfield JF, Doudna JA: **CRISPR-CasPhi from huge phages is a hypercompact genome editor**. *Science* 2020, **369**:333-337.
16. Faure G, Shmakov SA, Yan WX, Cheng DR, Scott DA, Peters JE, Makarova KS, Koonin EV: **CRISPR-Cas in mobile genetic elements: counter-defence and beyond**. *Nat Rev Microbiol* 2019, **17**:513-525.

17. Medvedeva S, Liu Y, Koonin EV, Severinov K, Prangishvili D, Krupovic M: **Virus-borne mini-CRISPR arrays are involved in interviral conflicts**. *Nat Commun* 2019, **10**:5204.
18. Koonin EV, Makarova KS, Wolf YI, Krupovic M: **Evolutionary entanglement of mobile genetic elements and host defence systems: guns for hire**. *Nature Reviews Genetics* 2019, **21**:119-131.
19. Frigols B, Quiles-Puchalt N, Mir-Sanchis I, Donderis J, Elena SF, Buckling A, Novick RP, Marina A, Penades JR: **Virus Satellites Drive Viral Evolution and Ecology**. *PLoS Genet* 2015, **11**:e1005609.
20. Chen J, Carpena N, Quiles-Puchalt N, Ram G, Novick RP, Penades JR: **Intra- and inter-generic transfer of pathogenicity island-encoded virulence genes by cos phages**. *ISME J* 2015, **9**:1260-1263.
21. de Sousa JM, Rocha EP: **To catch a hijacker: abundance, evolution and genetic diversity of P4-like bacteriophage satellites**. *Phil. Trans. R. Soc. Lond. B* 2021, **377**:1842.
22. Hays SG, Seed KD: **Dominant Vibrio cholerae phage exhibits lysis inhibition sensitive to disruption by a defensive phage satellite**. *Elife* 2020, **9**:e53200.
23. Dimitriu T, Szczelkun MD, Westra ER: **Evolutionary Ecology and Interplay of Prokaryotic Innate and Adaptive Immune Systems**. *Curr Biol* 2020, **30**:R1189-R1202.
24. Borges AL, Zhang JY, Rollins MF, Osuna BA, Wiedenheft B, Bondy-Denomy J: **Bacteriophage Cooperation Suppresses CRISPR-Cas3 and Cas9 Immunity**. *Cell* 2018, **174**:917-925.e910.
25. Landsberger M, Gandon S, Meaden S, Rollie C, Chevallereau A, Chabas H, Buckling A, Westra ER, van Houte S: **Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity**. *Cell* 2018, **174**:908-916. e912.
26. Chevallereau A, Meaden S, Fradet O, Landsberger M, Maestri A, Biswas A, Gandon S, van Houte S, Westra ER: **Exploitation of the cooperative behaviors of anti-CRISPR phages**. *Cell host & microbe* 2020, **27**:189-198. e186.
27. Rocha EP, Bikard D: **Microbial defenses against mobile genetic elements and viruses: Who defends whom from what?** *PLoS biology* 2022, **20**:e3001514.
28. LeGault K, Hays SG, Angermeyer A, McKitterick AC, Johura F-t, Sultana M, Ahmed T, Alam M, Seed KD: **Temporal shifts in antibiotic resistance elements govern virus-pathogen conflicts**. *Science* 2021, **373**:eabg2166.
29. Pasechnek A, Rabinovich L, Stadnyuk O, Azulay G, Mioduser J, Argov T, Borovok I, Sigal N, Herskovits AA: **Active Lysogeny in Listeria Monocytogenes Is a Bacteria-Phage Adaptive Response in the Mammalian Environment**. *Cell Rep* 2020, **32**:107956.
30. Carey JN, Mettert EL, Fishman-Engel DR, Roggiani M, Kiley PJ, Goulian M: **Phage integration alters the respiratory strategy of its host**. *Elife* 2019, **8**.
31. Altuvia S, Storz G, Papenfort K: **Cross-Regulation between Bacteria and Phages at a Posttranscriptional Level**. *Microbiol Spectr* 2018, **6**:RWR-0027-2018.
32. Silpe JE, Bassler BL: **A Host-Produced Quorum-Sensing Autoinducer Controls a Phage Lysis-Lysogeny Decision**. *Cell* 2019, **176**:268-280 e213.
33. Hernandez-Doria JD, Sperandio V: **Bacteriophage Transcription Factor Cro Regulates Virulence Gene Expression in Enterohemorrhagic Escherichia coli**. *Cell Host Microbe* 2018, **23**:607-617 e606.
34. Azulay G, Pasechnek A, Stadnyuk O, Ran-Sapir S, Fleisacher AM, Borovok I, Sigal N, Herskovits AA: **A dual-function phage regulator controls the response of**

cohabiting phage elements via regulation of the bacterial SOS response. *Cell Rep* 2022, **39**:110723.
35. Menouni R, Champ S, Espinosa L, Boudvillain M, Ansaldi M: **Transcription termination controls prophage maintenance in Escherichia coli genomes**. *Proc Natl Acad Sci U S A* 2013, **110**:14414-14419.
36. Pfeifer E, Hünnefeld M, Popa O, Frunzke J: **Impact of xenogeneic silencing on phage–host interactions**. *Journal of molecular biology* 2019, **431**:4670-4683.
37. Liu X, Lin S, Liu T, Zhou Y, Wang W, Yao J, Guo Y, Tang K, Chen R, Benedik MJ, et al.: **Xenogeneic silencing relies on temperature-dependent phosphorylation of the host H-NS protein in Shewanella**. *Nucleic Acids Res* 2021, **49**:3427-3440.
38. Doyle M, Fookes M, Ivens A, Mangan MW, Wain J, Dorman CJ: **An H-NS-like stealth protein aids horizontal DNA transmission in bacteria**. *Science* 2007, **315**:251-252.
39. Skennerton CT, Angly FE, Breitbart M, Bragg L, He S, McMahon KD, Hugenholtz P, Tyson GW: **Phage encoded H-NS: a potential achilles heel in the bacterial defence system**. *PLoS One* 2011, **6**:e20095.
40. Pfeifer E, Hünnefeld M, Popa O, Polen T, Kohlheyer D, Baumgart M, Frunzke J: **Silencing of cryptic prophages in Corynebacterium glutamicum**. *Nucleic Acids Research* 2016, **44**:10117-10131.
41. Williamson HS, Free A: **A truncated H-NS-like protein from enteropathogenic Escherichia coli acts as an H-NS antagonist**. *Mol Microbiol* 2005, **55**:808-827.
42. Bdira FB, Erkelens AM, Qin L, Volkov AN, Lippa Andrew M, Bowring N, Boyle Aimee L, Ubbink M, Dove Simon L, Dame Remus T: **Novel anti-repression mechanism of H-NS proteins by a phage protein**. *Nucleic Acids Research* 2021, **49**:10770-10784.
43. Markwitz P, Lood C, Olszak T, van Noort V, Lavigne R, Drulis-Kawa Z: **Genome-driven elucidation of phage-host interplay and impact of phage resistance evolution on bacterial fitness**. *The ISME journal* 2022, **16**:533-542.
44. Thomas R: **Control of development in temperate bacteriophages: I. Induction of prophage genes following hetero-immune super-infection**. *Journal of Molecular Biology* 1966, **22**:79-95.
45. Roux S, Krupovic M, Daly RA, Borges AL, Nayfach S, Schulz F, Sharrar A, Matheus Carnevali PB, Cheng JF, Ivanova NN, et al.: **Cryptic inoviruses revealed as pervasive in bacteria and archaea across Earth's biomes**. *Nat Microbiol* 2019, **4**:1895-1906.
46. Kirchberger PC, Martinez ZA, Luker LJ, Ochman H: **Defensive hypervariable regions confer superinfection exclusion in microviruses**. *Proc Natl Acad Sci U S A* 2021, **118**:e2102786118.
47. Refardt D: **Within-host competition determines reproductive success of temperate bacteriophages**. *ISME j* 2011, **5**:1451-1460.
48. Lemire S, Figueroa-Bossi N, Bossi L: **Bacteriophage crosstalk: coordination of prophage induction by trans-acting antirepressors**. *PLoS Genet* 2011, **7**:e1002149.
49. De Paepe M, Hutinet G, Son O, Amarir-Bouhram J, Schbath S, Petit M-A: **Temperate phages acquire DNA from defective prophages by relaxed homologous recombination: the role of Rad52-like recombinases**. *PLoS genetics* 2014, **10**:e1004181.
50. Botstein D: **A theory of modular evolution for bacteriophages**. *Ann N Y Acad Sci* 1980, **354**:484-490.
51. Mavrich TN, Hatfull GF: **Bacteriophage evolution differs by host, lifestyle and genome**. *Nature Microbiology* 2017, **2**:17112.

52. Dragoš A, Priyadarshini B, Hasan Z, Strube ML, Kempen PJ, Maróti G, Kaspar C, Bose B, Burton BM, Bischofs IB, et al.: **Pervasive prophage recombination occurs during evolution of spore-forming Bacilli**. *The ISME Journal* 2021, **15**:1344-1358.
53. Kupczok A, Neve H, Huang KD, Hoeppner MP, Heller KJ, Franz CMAP, Dagan T: **Rates of Mutation and Recombination in Siphoviridae Phage Genome Evolution over Three Decades**. *Molecular Biology and Evolution* 2018, **35**:1147-1159.
54. Moura de Sousa JA, Pfeifer E, Touchon M, Rocha EPC: **Causes and Consequences of Bacteriophage Diversification via Genetic Exchanges across Lifestyles and Bacterial Taxa**. *Mol Biol Evol* 2021, **38**:2497-2512.
55. Martinsohn JT, Radman M, Petit MA: **The lambda red proteins promote efficient recombination between diverged sequences: implications for bacteriophage genome mosaicism**. *PLoS Genet* 2008, **4**:e1000065.
56. Garriss G, Waldor MK, Burrus V: **Mobile antibiotic resistance encoding elements promote their own diversity**. *PLoS Genet* 2009, **5**:e1000775.
57. Köstlbacher S, Collingro A, Halter T, Domman D, Horn M: **Coevolving Plasmids Drive Gene Flow and Genome Plasticity in Host-Associated Intracellular Bacteria**. *Current Biology* 2021, **31**:346-357.e343.
58. Bailly-Bechet M, Vergassola M, Rocha E: **Causes for the intriguing presence of tRNAs in phages**. *Genome Res* 2007, **17**:1486-1495.
59. Yang JY, Fang W, Miranda-Sanchez F, Brown JM, Kauffman KM, Acevero CM, Bartel DP, Polz MF, Kelly L: **Degradation of host translational machinery drives tRNA acquisition in viruses**. *Cell Systems* 2021, **12**:771-779. e775.
60. Randich AM, Kysela DT, Morlot C, Brun YV: **Origin of a Core Bacterial Gene via Co-option and Detoxification of a Phage Lysin**. *Current Biology* 2019, **29**:1634-1646.e1636.
61. Pfeifer E, Moura de Sousa JA, Touchon M, Rocha EPC: **Bacteria have numerous distinctive groups of phage-plasmids with conserved phage and variable plasmid gene repertoires**. *Nucleic Acids Res* 2021, **49**:2655-2673.
62. Pfeifer E, A. Bonnin R, Rocha EPC: **Phage-plasmids spread antibiotic resistance genes through infection and lysogenic conversion**. *bioRxiv* 2022:2022.2006.2024.497495.
63. Fillol-Salom A, Martinez-Rubio R, Abdulrahman RF, Chen J, Davies R, Penades JR: **Phage-inducible chromosomal islands are ubiquitous within the bacterial universe**. *ISME J* 2018, **12**:2114-2128.
64. Netter Z, Boyd CM, Silvas TV, Seed KD: **A phage satellite tunes inducing phage gene expression using a domesticated endonuclease to balance inhibition and virion hijacking**. *Nucleic Acids Research* 2021, **49**:4386-4401.
65. Millman A, Melamed S, Leavitt A, Doron S, Bernheim A, Hör J, Lopatina A, Ofir G, Hochhauser D, Stokar-Avihail A: **An expanding arsenal of immune systems that protect bacteria from phages**. *bioRxiv* 2022.
66. Jaskólska M, Adams DW, Blokesch M: **Two defence systems eliminate plasmids from seventh pandemic Vibrio cholerae**. *Nature* 2022, **604**:323-329.
67. Chaikeeratisak V, Nguyen K, Khanna K, Brilot AF, Erb ML, Coker JKC, Vavilina A, Newton GL, Buschauer R, Pogliano K, et al.: **Assembly of a nucleus-like structure during viral replication in bacteria**. *Science* 2017, **355**:194-197.
68. Mendoza SD, Nieweglowska ES, Govindarajan S, Leon LM, Berry JD, Tiwari A, Chaikeeratisak V, Pogliano J, Agard DA, Bondy-Denomy J: **A bacteriophage nucleus-like compartment shields DNA from CRISPR nucleases**. *Nature* 2020, **577**:244-248.
69. Malone LM, Warring SL, Jackson SA, Warnecke C, Gardner PP, Gumy LF, Fineran PC: **A jumbo phage that forms a nucleus-like structure evades CRISPR–Cas DNA**

targeting but is vulnerable to type III RNA-based immunity. *Nature microbiology* 2020, **5**:48-55.
70. Selva L, Viana D, Regev-Yochay G, Trzcinski K, Corpa JM, Lasa I, Novick RP, Penades JR: **Killing niche competitors by remote-control bacteriophage induction.** *Proc Natl Acad Sci U S A* 2009, **106**:1234-1238.
71. Bruce JB, Lion S, Buckling A, Westra ER, Gandon S: **Regulation of prophage induction and lysogenization by phage communication systems**. *Curr Biol* 2021, **31**:5046-5051.e5047.
72. Brady A, Quiles-Puchalt N, Gallego Del Sol F, Zamora-Caballero S, Felipe-Ruíz A, Val-Calvo J, Meijer WJJ, Marina A, Penadés JR: **The arbitrium system controls prophage induction**. *Curr Biol* 2021, **31**:5037-5045.e5033.

Annotated references from the last few years

(•5) Systematic and quantitative view of the antiviral arsenal of prokaryotes

- A novel bioinformatic tool that detects anti-viral defense systems in prokaryotic genomes, it was used to analyse thousands of bacterial genomes, detecting a large diversity of anti-viral defense systems between and also within species.

(••8) Rapid evolutionary turnover of mobile genetic elements drives bacterial resistance to phages

- A genome-resolved cross-infection network of marine Vibrio identifies the genetic determinants of phage resistance. Resistance is encoded on putative MGEs that show fast evolutionary turnover and are akin to genomic defense islands. Such elements constitute >90% of the flexible genome and explain most of the differences in phage susceptibility among closely related bacterial strains.

(••10) Prophages mediate defense against phage infection through diverse mechanisms

- In this landmark work, the authors describe a variety of mechanisms through which prophages can prevent infection of their bacterial host by other phages. This study showed that repression of phage replication genes by resident prophages is not the most frequent mechanism of anti-phage defence, and other mechanisms, including modifications of the cell surface, represent more frequent molecular strategies of phage exclusion.

(••14) Phages and their satellites encode hotspots of antiviral systems

- Phage satellites are often regarded as parasites of phages. However, they can provide an advantage to their helper phages if they are able to increase its survivability. This is the case of the P4-like phage satellites of *E. coli* that, as discovered in this study, carry diverse anti-phage systems that target the phage competitors of their P2-like helpers.

(••24) Bacteriophage cooperation suppresses CRISPR-Cas3 and Cas9 immunity

- This study, together with Landsberger et al. (2018), shows that the inactivation of CRISPR-Cas immunity requires phage cooperation. This is one of the first documented examples of viral altruism where the production of Acr proteins by phage genomes that fail to replicate leave the cell immunosuppressed, which predisposes the cell for successful infection by other phages in the population.

(••25) Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity.

- See [24]

(•26) Exploitation of the Cooperative Behaviors of Anti-CRISPR Phages

- This study demonstrates that Anti-CRISPR (Arc) phages can be exploited by cheater non-Arc phages and illustrates how the strength of Arc proteins shape the evolutionary dynamics and social interactions of phage populations in natural communities.

(••28) Temporal shifts in antibiotic resistance elements govern phage-pathogen conflicts

- Using time-shift experiments, this study uncovered fluctuations in *Vibrio cholerae*'s resistance to phages in clinical samples. They show that diverse STX integrative and conjugative elements (ICEs) carrying both antibiotic resistance genes and anti-phage systems determine epidemic *Vibrio cholerae* susceptibility to phages. Remarkably, this study links phage and antibiotic resistances together on a single mobile genetic element, whose transfer is stimulated by phage infection.

(•29) Active Lysogeny in Listeria Monocytogenes Is a Bacteria-Phage Adaptive Response in the Mammalian Environment

- Unusual partnership between a pathogen and a prophage, where prophage integration/excision provides a genetic switch of a virulence program.

(•32) A Host-Produced Quorum-Sensing Autoinducer Controls a Phage Lysis-Lysogeny Decision

- Receptors of bacterial communication systems can be encoded by phages, which can use this to eavesdrop bacterial hosts and induce the lytic cycle at optimal host densities.

(••34) A dual-function phage regulator controls the response of cohabiting phage elements via regulation of the bacterial SOS response

- This study explores the mechanisms involved in the cross-regulation of two prophages on the same bacterium (*Listeria monocytogenes*). A regulator, AriS, has two domains that either regulate the phage genes or it downregulates the bacterial SOS response.

(•37) Xenogeneic silencing relies on temperature-dependent phosphorylation of the host H-NS protein in Shewanella

- A nice example on how bacterial regulation to environmental factors can affect the induction and re-integration of prophages, and thereby be used as a phenotypic switch.

(•42) Novel anti-repression mechanism of H-NS proteins by a phage protein

- A *Pseudomonas* lytic phage modulates the function of a bacterial encoded XS of the H-NS family. This has implications for gene regulation across the genome and is an example of how phages disrupt the host genetic network.

(•46) Defensive hypervariable regions confer superinfection exclusion in microviruses

- *Gokushvirinae*, a temperate phage of the understudied *Microviridae* family, prevent the injection of other microviruses through a hypervariable genomic region in their DNA pilot protein, an otherwise ancestral structural gene. This highlights the importance for phages to compete for hosts (and provide the latter with anti-phage defence mechanisms).

(•52) Pervasive prophage recombination occurs during evolution of spore-forming *Bacilli*

- Recombination between phages can change the phage lifestyle. Here, two prophages of *Bacillus subtilis* undergoing a sporulation selection regime, recombined to generate virulent phage hybrids.

(•53) Rates of Mutation and Recombination in *Siphoviridae* Phage Genome Evolution over Three Decades

- Recombination is thought to be pervasive in phages, but there is a lack of proper quantification of this phenomenon. This study provides estimates for substitution and recombination rates in a *Siphoviridae* phage over a 30-year period, showing that, relative to mutations, recombination contributes with much more polymorphism.

(•54) Causes and Consequences of Bacteriophage Diversification via Genetic Exchanges across Lifestyles and Bacterial Taxa

- By looking at pairwise gene similarity across thousands of phage genomes, this study quantified gene flow as a function of (and across) phage lifestyle, as well as the presence of molecular mechanisms potentially responsible for such changes.

(•60) Origin of a Core Bacterial Gene via Co-option and Detoxification of a Phage Lysin

- This study details the domestication of a phage lysin as part of a bacterial morphogenesis gene, SpmX.

(•61) Bacteria have numerous distinctive groups of phage–plasmids with conserved phage and variable plasmid gene repertoires

- Phages and plasmids are typically regarded as distinct entities. This study shows that phage-plasmids resemble both. It quantifies for the first time the abundance of these elements and suggests they may act as a genetic hub for exchanges between phages and plasmids.

(•64) A phage satellite tunes inducing phage gene expression using a domesticated endonuclease to balance inhibition and virion hijacking

- Recombination involving phages is not always beneficial. This study shows that an important component of the hijacking machinery of the PLE phage satellite, resulted from the acquisition and repurpose of a phage gene. Thus, recombination between mobile elements can actively contribute to their interactions.

(•66) Two defence systems eliminate plasmids from seventh pandemic *Vibrio cholerae*

- Multiple defence systems can cooperate to protect against mobile elements and can have widespread effects across multiple MGEs. That is the case for a defence system in *Vibrio* that prevents the spread of plasmids through bacterial populations and also the spread of phages.

(•71) Regulation of prophage induction and lysogenization by phage communication systems

- Both this study and another in the same issue [72] show how communication between prophages in different cells can allow them to coordinate their induction in conditions that are favourable for it.

(•72) The arbitrium system controls prophage induction

- See [71]