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Phage transmission strategies: are phages farming their host?

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Extensive coevolution has led to utterly intricate interactions between phages and their bacterial hosts. While both the (short-term) intracellular molecular host-subversion mechanisms during a phage infection cycle and the (long-term) mutational arms race between phages and host cells have traditionally received a lot of attention, there has been an underestimating neglect of (mid-term) transmission strategies by which phages manage to cautiously spread throughout their host population. However, recent findings underscore that phages encode mechanisms to avoid host cell scarcity and promote coexistence with the host, giving the impression that some phages manage to 'farm' their host population to ensure access to host cells for lytic consumption. Given the tremendous impact of phages on bacterial ecology, charting and understanding the complexity of such transmission strategies is of key importance.

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Introduction

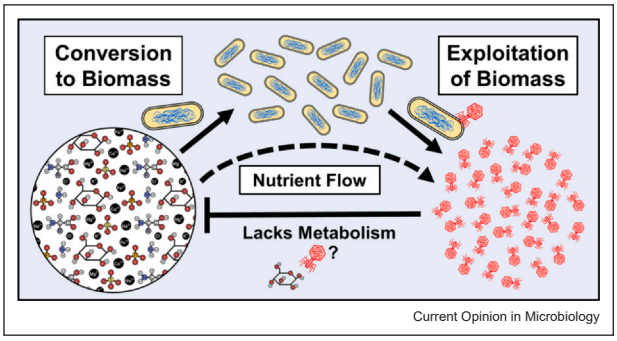
Bacterial viruses (also referred to as bacteriophages or phages) are ubiquitous in nature, and although they require a host cell to multiply, they often outnumber their hosts by an order of magnitude [1,2]. The life cycle and biology of phages has been extensively studied, and mainly became mapped along the lines of two distinct reproductive routes: lytic development (performed by both lytic and temperate phages) and lysogenic development (only performed by temperate phages) [3]. In case of lytic development, the incoming phage chromosome enforces its replication and the production of new phage particles that are typically released by lysing the host and that enable further horizontal transmission throughout the host population. During this lytic development, phage proteins typically hijack host machinery and resources for massive replication of phage chromosomes and production of capsid proteins [4,5]. In case of temperate phages, the incoming phage chromosome can alternatively decide to lysogenically convert its host, thereby establishing itself as a seemingly more dormant prophage. The latter is typically integrated into the host chromosome, where it becomes stably replicated and segregated to ensure further vertical transmission [6]. However, when the host cell suffers stress such as DNA damage, the prophage can manage to exit this quiescent state and enter the lytic cycle [7,8].

Despite the above-perceived paradigms of phage transmission dynamics, the function and necessity of many phage-encoded proteins still remain obscure [9,10], suggesting higher-than-anticipated complexities in the way phages deal with their host population. In this context, we would like to focus on the possible 'farming' capacity of phages, being their presumed ability to intentionally cultivate and harvest host cells. Indeed, since phages can only indirectly access the nutritional resources from their environment via the metabolism and biomass of their host cells (Figure 1), it seems intuitive that phages have evolved mechanisms and strategies to avoid host cell scarcity and/or secure a steady supply of host cells for lytic consumption. In fact, recent literature is increasingly yielding examples that tend to incline toward this perspective and that showcase the resourcefulness of phages managing to stably coexist with their host.

Lytic phages manage to avoid host scarcity

The idea of strictly lytic phages inevitably consuming all of their host cells within the niche (or forcing them toward phage resistance) tends to conflict with actual observations in nature where lytic phages are found to stably coexist with their susceptible host cells [11,12]. Obviously, the heterogeneity of some natural environments or the

Figure 1



Phages need to exploit bacterial cells to gain access to the nutritional resources of their environment. While bacteria can directly access nutrients and convert them to biomass, phages need their host as an essential go-between to turn resources into phage particles. It therefore seems intuitive that phages have evolved strategies to avoid host cell scarcity and/or secure a steady supply of host cells for lytic consumption. These ('farming') strategies range from deferring from lytic development when host cells have poor growth prospects, up to supporting the emergence of transiently nonsusceptible subpopulations of host cells that can be lytically consumed ('harvested') afterward.

presence of biofilms can often provide spatially structured refuges that can serve as a continuous sink of fresh host cells [13,14]. However, also in homogeneous environments lacking such refuges, many lytic phages manage to coexist with their host without the need of being caught up in a mutational arms race, indicating lytic phages must have genetically wired strategies to avoid host depletion as well.

Indeed, some lytic phages (such as coliphage T4) seem to be able to defer their lytic development when infecting a starved host that likely signals a nutritionally poor and unproductive environment [15,16]. In this context, the chromosome of *Bacillus subtilis* phage φ29 even encodes binding sites for the host Spo0A protein (being the master regulator triggering the sporulation pathway) in order for its lytic cycle to become repressed and postponed when a hostile environment urges its host to commit to sporulation [17]. In the same vein, ΦCbk-like phages of *Caulobacter* species seem to be able to monitor the cell-cycle state of its host, thereby distinguishing between its stalked versus swarmer phenotype. More specifically, swarmer cells of *Caulobacter* are characterized by low levels of activated CtrA developmental master regulator that coincide with active flagella and pili that are recognized by ΦCbk-like phages to infect the cell. The phage chromosome, however, contains several CtrA-binding sites that are predicted to synchronize the cell-cycle state with lysis timing. High levels of active CtrA might trigger lysis and are only achieved when the swarmer cell switches to the stalker state in suitable environments. As such, the phage seems to defer lysis of the nondividing and more likely to be a solitary swarmer cell up until this cell differentiates into a stalker cell under conditions that support a higher density of host cells [18].

Alternatively, some lytic phages seem to foster the emergence of a transiently resistant subpopulation that serves as a feedstock for lytic consumption later on. For example, *Bacteroides intestinalis* phage ΦcrAss001 can only infect host cells with specific capsular polysaccharides (CPS). Since *B. intestinalis* displays stochastic phase variability in its CPS modifications, only the fraction of cells with susceptible CPS becomes lytically consumed, while cells with nonsusceptible CPS are left unharmed. These nonsusceptible cells can therefore grow out as a transiently resistant population that stochastically keeps spawning off susceptible cells that can be lytically consumed by ΦcrAss001 [19]. Along similar lines, a more elaborate and circular use of phase variation was recently demonstrated with Fletchervirus F358, which uses its receptor-binding protein 1 (RBP1) to bind to specific CPS of its *Campylobacter jejuni* host. In this case, it was found that *C. jejuni* likewise displays stochastic phase variability in the modification of its CPS, leading to OFF/ON switching behavior of F358's receptor [20]. As such, infection by Fletchervirus F358 results in the lysis of receptor^{ON} cells (targeted by RBP1 of phage F358), which in turn allows receptor^{OFF} cells to take over the host population. However, phage F358 itself also displays phase variation in the expression of an alternative RBP2. If F358 has an active RBP2, it can circumvent the need for the CPS modification and infect and lyse receptor^{OFF} cells, leading receptor^{ON} revertants to take over the population [20]. It could perhaps be argued that many lytic phages have throughout evolution been selected to reach for phase- variable host receptors, as this would allow them to prevent host eradication and steer toward coexistence with their host.

Interestingly, the massive lysis of susceptible host cells on itself can also be a trigger toward securing a reserve population that can be lytically harvested later on. In an *Enterococcus faecalis* population, for example, massive lysis by phage Efs7 leads to the accumulation of phage-encoded endolysins in the environment, which in turn forces uninfected survivors into cell wall-deficient L-forms that lack phage receptors associated with the cell wall [21]. As such, these L-forms proliferate and remain phenotypically resistant to the phage until resynthesis of their cell wall can be reinitiated later on [21]. Similarly, upon phage SPP1 infection of its *Bacillus subtilis* host, a yet-to-be-determined signal is released and transmitted to neighboring noninfected cells that triggers expression of the host-encoded SigX sigma factor. SigX in turn activates the *dlt* operon, which encodes enzymes that modify the phage receptor (i.e. wall teichoic acids) and thereby raise a transiently resistant subpopulation [22]. As such, a reservoir of transiently uninfectable cells is

formed that has the ability to revert back to an infectable state. Moreover, lysis by this same phage was also suggested to facilitate the spread of receptor-containing membrane vesicles that can fuse with nonsusceptible cells and hence make them susceptible for infection as well. As a result, massive lysis can even expand the host range toward previously unsusceptible cells [23].

Temperate phages manage to defy stable lysogeny

Contrary to lytic phages, temperate phages are assumed to have found the solution to host scarcity by integrating into the host chromosome as a prophage. But even though establishment of lysogeny is often seen as the path toward a stable vertical transmission and coexistence with the host, important phage (lytic) capacities escape selection and might become genetically eroded during lysogeny, as is evident from the countless examples of prophage remnants in bacterial genomes [24,25]. However, recent findings support the notion that lysogeny might be overrated as the (only) go-to strategy for temperate phages faced with host scarcity.

The classic coliphage Lambda model suggests that temperate phages tend to use a high multiplicity-of-infection (i.e. how many phage chromosomes simultaneously infect the same cell) as a proxy for imminent host depletion, and therefore as a cue to change the reproductive strategy from horizontal (lytic) toward vertical (lysogenic) transmission [26]. However, microscopic scrutiny of the dynamics of the temperate lambdoid P22 phage infecting its *Salmonella* Typhimurium host led to the observation that host scarcity can also lead to the establishment of a phage carrier state in which a polarly localized P22 episome is formed that segregates asymmetrically among daughter cells [27]. Although the cell inheriting the P22 episome eventually becomes lysogenized, its former P22-free daughter cells nevertheless cytoplasmically inherit superinfection exclusion factors (SEFs) produced by the P22 episome. This allows a transiently resistant P22-free subpopulation to emerge that — upon the gradual cytoplasmatic dilution of these SEFs — again becomes susceptible to P22 infection. However, because of this gradual SEF dilution, the number of incoming P22 phages is heavily restricted, thereby relaying a high phage-to-host ratio (normally favoring establishment of lysogeny) into a low multiplicity-of-infection (favoring lytic consumption) [28]. Because of these carrier state and SEF dynamics, P22 seems to be able to partly defy lysogeny by continuously raising and lytically harvesting subpopulations of host cells.

In the same context, other temperate phages seem to have evolved intricate mechanisms to *wake up in time* and reverse lysogeny when new susceptible host cells are detected and before potential crippling mutations accumulate. In this context, it was already previously discovered that SPbeta phages infecting *Bacillus subtilis* make use of a particular quorum sensing mechanism (rather than the multiplicity-of-infection) to switch from horizontal to vertical transmission. Indeed, during lytic consumption of a large part of the host population, a phage-encoded small peptide (AimP, also referred to as arbitrium peptide) progressively accumulates in the environment and eventually signals the remaining infections to incline toward establishment of lysogeny [29]. However, most recently, this system was also shown to affect the decision-making of the subsequently established SPbeta prophages. More specifically, at high lysogen concentrations, the accumulation of arbitrium peptide produced by the prophage will block the host DNA damage response and therefore restrict prophage induction. However, in environments where the lysogen again becomes surrounded by susceptible (nonlysogen) host cells, the correspondingly diluted arbitrium concentration will eventually enable the DNA damage response to activate prophages and release phage particles that can commit to lytic consumption of susceptible host cells [30–32]. As such, prophage dormancy can be alleviated as soon as new susceptible host cells arise that can again fuel horizontal transmission.

Alternatively, some prophages rather eavesdrop on host-derived quorum sensing signals for their wake up call [33,34]. As such, phage ARM81ld was found to sense the C4-HSL quorum signal of its *Aeromonas* host by using a phage-encoded LuxR homolog to decide upon prophage induction [33–35]. Furthermore, ARM81ld also seems to estimate the nutritional competition encountered by its host, since ARM81ld is also able to detect the C8-HSL quorum sensing signal of *Vibrio fischeri* cells (that it cannot infect). In fact, in cases where *Aeromonas* is being outcompeted by *V. fischeri*, ARM81ld will sense the high C8-HSL concentration and — given the poor prospects of its host — refrain from induction [35].

Finally, it is worth underscoring that filamentous phages have technically found a very fruitful merge between horizontal and vertical transmission. Indeed, their ability to continuously replicate within and egress from their host cell without actually lysing it by default circumvents host cell scarcity and secures continuous coexistence [36].

Conclusion

Many examples support the notion that phages are not only able to subvert individual host cells, but actually succeed in managing their host at the population level. Nevertheless, it still remains to be further established

whether all of the provided examples represent intentional strategies to secure coexistence. Indeed, while some mechanisms clearly appear to be supported by phage-borne functions, other dynamics might rather stem from intrinsic host cell behavior for which it is unclear whether it is truly exploited by the phage. However, with host cells as the essential intermediary and catalyst toward turning an environment's resources into phage particles, strategies to prevent host scarcity and raise or exploit transiently resistant subpopulations (i.e. 'farming' capacity) could well have been positively selected throughout evolution.

Typically, the mechanisms underlying these possible farming strategies depend on often subtle and transient interactions that need to be interpreted over different scales. In fact, biochemical interactions need to be put in a cellular context and variations (both cell-to-cell and over time) in individual cellular behavior need to be integrated at the level of populations in order to understand the relevant emergent properties and how they impact infection dynamics at large. For this, the live visualization, monitoring, and comparison of different phage–host interactions inside and between large numbers of cells within an infected population will become increasingly important, and will require further advancements in fluorescent reporter engineering (in both phages and bacteria) and fluorescence microscopy approaches. Finally, these efforts should further scale up to also take into account the full complexity imposed by ecologically relevant settings, such as differently structured microenvironments and the presence of various phages competing for the same host.

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Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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