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Phage–host interactions during pseudolysogeny

Lessons from the Pid/*dgo* interaction

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Although the study of phage infection has a long history and catalyzed much of our current understanding in bacterial genetics, molecular biology, evolution and ecology, it seems that microbiologists have only just begun to explore the intricacy of phage–host interactions. In a recent manuscript by Cenens et al. we found molecular and genetic support for pseudolysogenic development in the *Salmonella* Typhimurium–phage P22 model system. More specifically, we observed the existence of phage carrier cells harboring an episomal P22 element that segregated asymmetrically upon subsequent divisions. Moreover, a newly discovered P22 ORFan protein (Pid) able to derepress a metabolic operon of the host (*dgo*) proved to be specifically expressed in these phage carrier cells. In this addendum we expand on our view regarding pseudolysogeny and its effects on bacterial and phage biology.

Keywords: phage carrier state, pseudolysogeny, phage–host interactions, *Salmonella* Typhimurium, phage P22

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Insights in Pseudolysogeny and the Phage Carrier State

The two best described and understood routes in phage propagation are the lytic and lysogenic cycle. During the lytic cycle, phages will take advantage of the host cell to extensively replicate their DNA and package it in viral capsids. In most cases, sudden cell lysis accompanies the release of several hundreds of new phage particles.[1,2] In contrast, temperate phages also have the ability to lysogenize their host as a prophage by integrating their chromosome into that of the host. During lysogeny the phage genome is therefore stably replicated in synchrony with the hosts replication cycle.[3,4] Prophages can again be activated into the lytic cycle by environmental factors causing stress in the host cell. In fact, many prophages respond to activation of the host's DNA damage (SOS) response, which provides the necessary trigger to relieve prophage repression and escape from their troubled host.[5]

However, in addition to this classical bifurcation into either lytic or lysogenic propagation, pseudolysogeny has been proposed as an alternative developmental route.[6-9] Early interest in pseudolysogeny stemmed from the observations of postponed cell lysis by phages in nutrient-depleted hosts. Interestingly, phage production and cell lysis proceeded immediately when a spike of nutrients was added to such starved hosts,[10,11] leading to the idea that phages can be carried inside the host without commitment to either lytic or lysogenic proliferation. This phage carrier state was defined by Ripp and Miller as "a phage–host cell interaction in which the nucleic acid of the phage, upon infection of an appropriate host cell, neither establishes a long-term, stable relationship (i.e., lysogeny) nor elicits a lytic response."[10] Obviously, given our currently poor molecular genetic knowledge on the phenomenon of pseudolysogeny, this definition is bound to be subjected to further refinement.

In a recent manuscript,[12] using *Salmonella* Typhimurium and its temperate phage P22 as a model system together with time-lapse fluorescence microscopy as a tool to study phage infection dynamics at single cell resolution, we were able to

fluorescently track the intracellular whereabouts of the phage chromosome and for the first time visually observed the emergence of stable phage carrier cells (PCCs) in an infected population. More specifically, PCCs were found to carry one (or possibly a complex of) unintegrated P22 chromosome(s), and this stable episomal P22 element became asymmetrically segregated upon subsequent divisions. As a direct consequence, the observed PCC state became inherited by only one of the emerging siblings, which is in striking concordance with the very early findings of Zinder[13] and Levine and Schott.[14] Using population-level approaches, these authors proposed the segregation of P22 sensitive cells from a P22 infected cell destined to become lysogenized, hypothesizing that a pseudolysogenic state had to exist that could give rise to lysogens and non-lysogens.

This asymmetric segregation of the P22 episome is in sharp contrast to the behavior of other known stable phage episomes that actually make use of elaborate symmetrical segregation and post-segregational killing mechanisms to ensure proper partitioning and maintenance in host cell siblings. A well-known example of the latter is phage P1, which exists as a circular episomal fragment and ensures the proper segregation of two P1 genomes by an ATP-dependent partitioning system composed of a specific *parS* sequence and ParA and ParB proteins.[15,16] This partitioning system is further sustained by P1-borne expression of a stable toxin (Doc) and its rapidly degraded antitoxin (Phd). This toxin-antitoxin complex functions as an addiction module that leads to cell death in siblings that would lose the P1 chromosome (i.e., post-segregational killing), since they are unable to replenish the antitoxin and succumb to the lethal action of the liberated toxin.[17]

As a distinct and possibly transient developmental route, the phage carrier state might confer a number of conditional advantages to the phage. In fact, Ripp and Miller[10,11] hypothesized that it might be beneficial for phages (especially obligately lytic ones) to reside in the bacterial host to protect their DNA-against the harsh conditions outside the host. In fact, physicochemical factors such as UV-light, pH and temperature can drastically reduce the half-life of virions.[18] In addition, it could also be a mechanism to overcome a starved host by preventing an abortive replication or integration event due to lack of resources. Similarly, another advantage of behaving as a pseudolysogen (instead of a real lysogen) might be the ability of the temperate phage to prevent being entirely dependent on the host's DNA damage response to escape from hibernation. Indeed, although spontaneous induction of prophages does occur in lysogens once every 10^5–10^8 cells,[19] bacterial numbers are often lower in environmental settings, indicating that spontaneous prophage induction would not always provide an adequate escape route.

Clearly, the presence of PCCs together with cells undergoing lytic and lysogenic development adds to the dynamic complexity of phage infections in the environment, and might have important ecological repercussions. As such, this phenomenon could explain why such high phage titers are found in environments where most of the time bacterial growth is low and unsupportive for massive phage production due to lack of nutrients.[10,11] Moreover, pseudolysogenic behavior might also prevent phages to be detected by traditional plating and plaquing methods, leading even to an underestimation of their prevalence and diversity.

The Increasing Intricacy of Phage–Host Interactions

In support of their proliferation, phages have evolved to interfere with the physiology of their host in a multitude of ways. Obviously, most of the currently described phage–host interactions fit within the typical lytic or lysogenic mode of propagation. Indeed, during lytic proliferation a large number of dedicated interactions occur to redirect host cell machinery and resources toward massive phage replication and capsid production. Corresponding examples stemming mainly from the well-studied lytic T4 and T7 phages include hijacking the host RNA-polymerase complex to ensure phage transcription, disabling phage restriction systems, inhibiting protease activity, and specific modifications of host chaperones which allow proper folding of specific phage proteins.[20,21] Furthermore, several phages infecting cyanobacteria express photosynthesis genes to boost the photosynthetic machinery of their host during infection.[22]

In contrast, prophages are dependent on their hosts' well-being to survive. Therefore they often provide the host cell with additional benefits to compensate for the extra genomic baggage they impose. Apart from causing immunity against infection with related phages, adaptive genomic arrangements[23] and horizontal gene transfer,[24] prophages tend to encode numerous additional genes conferring a beneficial effect on the physiology of their host.[23,25,26] While research has historically focused on prophage-borne virulence genes[26] (such as the cholera toxin in *Vibrio cholera*,[27] the shiga toxin in STEC *E. coli* strains[28] and the *SopE* gene in *Salmonella enterica*[29]), it is becoming increasingly evident that prophages can affect the ecological fitness of their host as well. In this context, it has indeed been shown that lysogenic phages have an important role in multiple aspects of the life cycle of *Bacillus anthracis*, including sporulation and biofilm formation.[6]

In our study, we also observed a peculiar phage–host interaction to be mounted specifically in PCCs. Indeed, only the latter cells experienced the expression of a newly discovered ORFan locus on the P22 genome.[12] This locus was termed *pid* (P22 instigator of *dgo*-expression) since its geneproduct (Pid) specifically derepresses the *dgo*-operon of the host. Although the regulatory aspects enabling *pid* expression to be dedicated to the phage carrier state so far remain elusive, our observations might be indicative of the presence of other phage-borne genes whose timing of expression and role could be intended for the phage carrier state.

The reason why Pid would specifically target galactonate metabolism and whether or not this interaction is beneficial for the phage and/or the host so far remains unclear. Nevertheless, the *dgo* operon was previously found to be important in virulence and intracellular survival,[30,31] and was also found to be controlled by the

PmrAB two component transduction system, which mediates resistance to antimicrobial peptides commonly produced by cells of the innate immune system.[32]

Interestingly, thus far only a few phage proteins have been identified which directly influence host gene expression. In this context, the small TorI protein encoded by the defective KplE1 prophage in *Escherichia coli* K12 was previously shown to downregulate the host *torCAD* operon, which is involved in the anaerobic respiration of trimethylamine N-oxide (TMAO) and is controlled by the two component system TorS/TorR.[33] More specifically, although TorI is essentially a recombination directionality factor,[34] it is also able to bind the TorR response regulator at its effector site, possibly preventing RNA-polymerase recruitment to the *torCAD* operon.[33] A related example concerns the secondary activity of the CI repressor from phage λ and several other phages, including P22. Although this well-known repressor has a main function in establishing and maintaining the prophage state,[35] micro-array data revealed that CI also represses the *E. coli pckA* promotor.[36] The pckA gene encodes the phosphoenolpyruvate carboxykinase, and is part of the gluconeogenesis pathway, which allows growth on succinate among other carbohydrates.[36] Most interestingly, next to a binding site for the λ CI, the *pckA* promotor region also has different homologs binding sites for the CI repressor of P22 and three other phages.[36] By preventing gluconeogenesis in an established lysogen, this interaction drastically lowers growth rate on succinate. Although it has been proposed in this case that a slower growth rate could shield the lysogen from immune system mediated killing,[36] it generally remains enigmatic why phages would metabolically interfere with their host during non-lytic (i.e., as a lysogen or pseudolysogen) development.

Finally, it is worth noting that a *pid* allele identical to the one in P22 is also present in the sub-Saharan *Salmonella* Typhimurium D23580 strain, which is notorious for causing systemic infections in humans.[37] Within this strain *pid* resides in an uncharacterized lysogenic phage which has multiple homologous modules with P22, including the region containing *pid*. Most recently, however, a homolog of *pid* was also found in a prophage of the newly sequenced *Enterobacter cloacae* subsp *cloacae* ENHKU01 strain.[38] Interestingly, in both cases the *pid* locus is oriented opposite to the late genes such as in P22,[12] although their impact on host cell physiology is still under study.

Conclusions and Further Perspectives

Developmental paths in phage biology that deviate from classical lytic or lysogenic proliferation have long remained overlooked and cryptic. The advent of novel cell biology approaches enabling the live visualization and interrogation of phage infected populations at single cell resolution will greatly contribute to our comprehensive understanding of the complex dynamics and heterogeneity unfolding in such populations, and thereby provide insights in features that often escape proper detection and analysis with current omics-technologies.

Our results add to the current definition of pseudolysogeny, as this state proves also to exist in an actively growing host cell and to support novel phage–host interactions, as opposed to being an inert or idle representation of the phage. Future studies will have to molecularly authenticate the presence of PCCs in other infection models, while the molecular events and environmental cues orchestrating and supporting the phage carrier state and pseudolysogenic development should be further identified to increase our understanding of this phenomenon as well as its impact on phage ecology. In conclusion, our results underscore that phage–host interactions have an unsurpassed intricacy with regard to timing and populational distribution, and are likely to be more prevalent than currently realized.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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