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.

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 and Levine and Schott. 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. 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.

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 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, the action of the liberated toxin.17

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(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 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.35 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 promoter.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 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 promoter.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 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 promoter.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 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 promoter.36

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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