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Spotlight

Structure Regulates Phage Lysis–Lysogeny Decisions

Jimmy T. Trinh[1,2] and Lanying Zeng[1,2,*]

There are many strategies by which cell fates are decided. In one intriguing case, viruses communicate via the quorum-sensing-like 'arbitrium' system to bias infection outcomes. Through elucidating the detailed molecular mechanisms of such strategies, we can better understand viral propagation and offer insights into the treatment of viral diseases.

Cellular decision-making is a ubiquitous and vital process for all living systems, and guides development and disease in organisms. Therefore, it is of paramount importance to characterize the detailed mechanisms of how cell fate selection occurs. The lysis–lysogeny decision that bacteriophages ('phages') make upon infecting their bacterial host represents one of the simplest decisions occurring in nature. Temperate phages choose between lysis, a lifestyle featuring the production and release of viral progeny via host destruction, and lysogeny, a dormant lifestyle where the virus propagates along with its host. Complex decision-making behaviors have recently been discovered in different phage systems, including intracellular individuality and interactions [1,2], non-binary fate outcomes [3,4], and small-molecule communication between generations of phages [5]. Regarding the communication behavior, mechanistic details are relatively scant.

Bacillus phages phi3T and SPbeta produce different 'arbitrium' peptides during infection, which are processed by their hosts and released in their mature form into the environment, as reported by Erez *et al.* [5]. These mature six amino acid peptides are then imported into neighboring cells. If these cells become infected, the peptides bind to AimR, which controls the expression of *aimX*, to bias the cells toward lysogeny. This overall process can be interpreted as viruses of one generation informing later generations of host scarcity, particularly at high levels of the peptide. The features of this system have also been identified in many other *Bacillus* phages. This motivates investigation of the detailed mechanisms of strategies for communication and consequent decision-making.

In a newly published paper in *Nature Microbiology*, Dou *et al.* focused on characterizing and comparing the action of different communication peptides from a structural perspective [6]. One key observation was that the different phages (phi3T and SPbeta) with different peptides (SAIRGA and GMPRGA, respectively) appeared to influence the lysis–lysogeny decision to different degrees. This suggests that there are differences in the molecular mechanisms of their action. Notably, phi3T and SPbeta both infect *Bacillus subtilis*, but were used to infect different strains by Erez *et al.* and Dou *et al.* because some *B. subtilis* strains carry the SPbeta prophage, making them immune to SPbeta infection. AimR was reported to activate *aimX* to promote lysis, likely by binding to phage DNA as a transcriptional activator, because the lysogeny-inducing, AimR-binding peptides decreased *aimX* transcription [5]. Dou *et al.* explored the effects of the peptide binding to AimR by crystallizing AimR from different phages, finding a

dimeric AimR for both phi3T and SPbeta in the absence of peptide binding. In the presence of their peptides, the AimR proteins of different phages exhibit different conformational changes. Phi3T AimR (phAimR) turned from dimeric to monomeric in the presence of its peptide, SAIRGA. By contrast, GMPRGA, the peptide of SPbeta, stabilized the dimeric state of SPbeta AimR (spAimR), consistent with another structural study of spAimR published earlier suggesting that AimR regulation by the SPbeta peptide does not involve AimR monomerization [7].

In addition, the SPbeta peptide appeared to promote lysogeny to a lesser extent than the phi3T peptide [6]. This suggests that the aforementioned differences in AimR alteration allowed modulation of lysogeny promotion (or lysis inhibition), where AimR dimer disruption might inhibit lysis more strongly for phi3T. By disrupting phAimR dimerization, the phi3T peptide strongly inhibits the ability of phAimR to activate *aimX*, whereas, by stabilizing the spAimR dimer, the SPbeta peptide carried out a similar function. In the mutation/complementation analysis, the residues and regions important for AimR dimerization were identified, showing that, in the absence of dimerization, lysis was impaired with or without the peptide, and in both phi3T and SPbeta. By mutating the peptide-binding region of AimR, the paper showed that blocking peptide binding decreases the likelihood of lysogeny for both phages. From these results it is clear that the phi3T peptide induces a monomeric form of phAimR to promote lysogeny. For SPbeta, the results suggest that, although full abrogation of the spAimR dimer can promote lysogeny, the SPbeta peptide actually locks spAimR in its dimeric form to promote lysogeny. That the SPbeta peptide regulates decision-making by stabilizing the spAimR dimer, even though this could also be accomplished by dissolving the dimer, demonstrates that there is more complexity to this specific system than simple differences in oligomerization states.

It is important to note that SPbeta lysogeny may be enhanced more by the SPbeta peptide than phi3T lysogeny is enhanced by its respective peptide, as reported by Erez *et al.* [5]. These data differ from the study by Dou *et al.* [6], where SPbeta lysogeny was relatively impaired compared to phi3T, particularly without the external addition of the synthesized SPbeta peptide. Recall that different strains were used for infection with SPbeta – BEST7003 and CU1050 for the Erez *et al.* and Dou *et al.* studies, respectively. CU1050 carries a potentially relevant ochre-suppressor [8,9], and was derived distinctly from BEST7003 [10]. Regardless, it is clear that divergent biological behaviors can arise from subtle structural changes. Moving forward, it would be helpful to consider the strain backgrounds for similar studies, and this would aid in the interpretation of experimental results.

Overall, these types of studies which directly target the elucidation of detailed mechanisms are extremely valuable. Phage decision-making systems such as those of phi3T and SPbeta are not yet well characterized in terms of full genetic pathways and host interactions, generally limiting comprehension of these systems, and thus their potential applications. Therefore, it is exciting to see that Dou *et al.* have devoted effort toward revealing the mechanistic details of how decision-making is regulated by comparing the structural differences between these systems, where small changes in protein structure can shape the fates of entire cells and communities.

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Spotlight

Building Walls: Work That Never Ends

Helene Botella[1] and Julien Vaubourgeix[1,2,*]

Fluorescent amino acid analogs have proven to be useful tools for studying the dynamics of peptidoglycan metabolism. García-Heredia and colleagues showed that their route of incorporation differs depending on the adjunct fluorophore and applied this property to investigate mycobacterial peptidoglycan synthesis and remodeling with heightened granularity.