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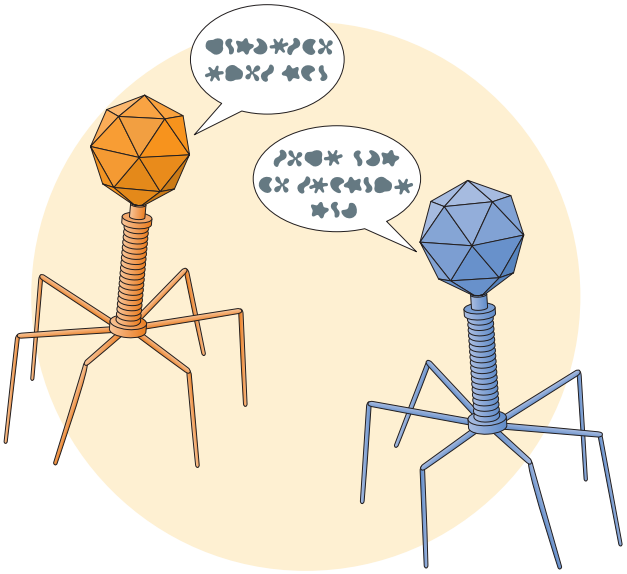
VIRAL INFECTION

The language of phages

> phages use the arbitrium communication system to decide whether to enter the lytic or lysogenic life cycle

Phages exhibit two distinct life cycles in bacteria, a lytic cycle and a lysogenic cycle. During the lytic cycle, phages replicate and progeny particles are released through lysis. By contrast, during lysogeny, phages integrate their genomes into the bacterial chromosome and enter a dormant state. At a later stage, such temperate dormant phages can re-enter a lytic cycle and release progeny. Now, Erez, Steinberger-Levy *et al*. report that phages of *Bacillus* species use a peptide-based communication system to decide whether to enter the lytic or lysogenic life cycle.

This discovery was fortuitous, as the authors initially looked for molecules that were secreted by phage-infected bacteria. They prepared conditioned media from cultures of bacteria that were infected with the phage phiT3 and filtered it to remove bacteria and phages, and to retain small molecules. *Bacillus subtilis* cells that were infected and grown in the conditioned medium seemed to be more resistant to lysis than cultures grown in control media.



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This intriguing result suggested that a small molecule is released during infection by phiT3 and that this molecule affects the lysis–lysogeny decision.

During quorum sensing, which is a form of cell–cell communication that is used by bacteria to coordinate gene expression, signalling molecules are released and imported into bacteria by oligopeptide permease (OPP) transporters. To test the involvement of OPPs, the authors deleted an essential subunit of an OPP transporter in bacteria. Infected mutant bacteria that were grown in the conditioned medium showed increased lysis compared with the wild type. This suggested that the peptide that is released into the medium and taken up by OPP affects the lysis–lysogeny decision. The putative communication molecule was termed arbitrium. Indeed, semi-quantitative PCR confirmed increased lysogenic infections in bacteria that were infected with phiT3 and grown in the conditioned medium compared with the control medium.

Sequencing the phiT3 genome revealed a candidate gene (*aimP*) that had features of *Bacillus* spp. quorum sensing peptides, which are processed by extracellular proteases after the secretion of a pre-peptide. The phage-encoded protein, which is 43 amino acids long, had an amino-terminal signal sequence and a carboxy-terminal consensus cleavage sequence, and mass spectrometry confirmed the presence of a mature peptide that was six amino acids long in the conditioned medium. Supplementing fresh medium with the isolated mature peptide resulted in decreased lysis of phage-infected bacteria compared with infected bacteria that were grown in control medium, or cells that were grown in medium that contained a quorum sensing peptide produced by bacteria. Next, the authors identified the gene upstream of *aimP* as an intracellular receptor for the arbitrium peptide, which they termed *aimR*. AimR was shown to bind to phage DNA as a dimer, and DNA binding was blocked when arbitrium was present. The authors proposed that the binding of arbitrium to AimR causes the receptor to monomerize, thus preventing its association with DNA. RNA sequencing (RNA-seq) revealed that AimR specifically regulates the expression of one transcript (termed *aimX*); transcript levels of *aimX*, which is a non-coding RNA, were high in the absence of the arbitrium peptide, but decreased when the peptide was present or when *aimR* was deleted. Moreover, deletion of *aimR* or *aimX* led to increased lysogeny. Together, these results suggest a model whereby, *aimR* and *aimP* are expressed following infection, and AimR induces the expression of *aimX*, which, in turn, promotes the lytic life cycle, possibly by acting as a regulatory non-coding RNA. AimP is secreted into the extracellular environment and cleaved. After several cycles of infection, the arbitrium peptide accumulates in the environment and is taken up by the OPP transporter. Inside bacteria, the peptide binds to and inhibits AimR, which leads to lysogeny.

In summary, this study shows that phages use the arbitrium communication system to decide whether to enter the lytic or lysogenic life cycle. The finding that different phages encode homologues of this system with different peptides suggests that phages use a sequence-specific molecular language.

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